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## ERRATA AND AUTHORS' EMENDATIONS

- Page 57, Table II, column 5, line 4, should read "BBSs" instead of "BBSs."
- Page 59, line 13 from bottom, should read "produced in 1921 were" instead of "produced in 1921 ere."
- Page 90, table, heading of first box should read "r=" instead of "r=."
- Page 98, line 2, should read "mosaic" instead of "mosiac."
- Page 159, line 1, should read "and fig. 2." instead of "and fig. 3."
- Page 159, next to last line, should read "(fig. 2)" instead of "(fig. 3)."
- Page 160, line 29, should read "(fig. 3)" instead of "(Fig. 6)."
- Page 170, last line should read "in 0.5 per cent hydrochloric acid" instead of "in 0.5 per cent hydrochloric acid."
- Page 216, line 25, should read "(s)" instead of "(p)."
- Page 227, ftm. 4, last line, should read "v. 33, No. 1, p. 20" instead of "v. 33, p. 20."
- Pages 247-265, running head should read "*Digestibility of Treated Grain Hulls*" instead of "*Digestibility of Treated Grain Hulls.*"
- Page 246, line 6 from bottom, should read "aging" instead of "ageing." Line 8 from bottom should read "adsorption" instead of "absorption." Line 9 from bottom should read "adsorbable" instead of "absorbable." Line 10 from bottom should read "adsorbed" instead of "absorbed."
- Page 247, line 9, should read " $C_{48}H_{44}O_{10}$ " instead of " $C_{48}H_{40}O_{10}$ ."
- Page 255, Table II, head, should read "Water-soluble acidity" instead of "water-soluable acidity."
- In Plate 2, facing page 411, figures E and F should be interchanged.
- Page 428, line 7 from bottom, should be "In seven of these" instead of "In eight of these."
- Page 437, lines 17-18, should read "within 2 degrees" instead of "within narrow limits."
- Page 442, line 19, should read "each variety" instead of "each variety."
- Page 448, line 3, should read "China, India, South Africa," instead of "China, South Africa."
- Page 451, ftm. 2, last line, should read "notes in the summer of 1922" instead of "in June, 1922."
- Page 504, line 40, should read "conclusive" instead of "cionclusive." Line 41-42 should read "differentiation" instead of "differentiation."
- Plate 8, facing page 596, legend, line 1, should read "brachytic maize  $\times$  teosinte, natural size," instead of "brachytic maize  $\times$  teosinte."
- Page 718, line 20, should read "(nitrogen 0.046 per cent)" instead of "(nitrogen 0.068 per cent)." Line 25 should read "and treble superphosphate" instead of "and superphosphate."
- Page 755, last line, last word, should read "structures" instead of "structurer." Next to last line, last word, should read "or" instead of "os."
- Page 768, line 33, should read "Bureau of Chemistry" instead of "Bureau of Plant Industry."
- Page 815, line 27, should read "Figure 2" instead of "Figure 6."
- Page 837, bottom, should read "Key No. 375" instead of "Key No. 376."
- Page 843, line 9, should read "single pycnospore" instead of "single ascospore."



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No. 1

## PHYSIOLOGICAL STUDIES ON APPLES IN STORAGE<sup>1, 2</sup>

By J. R. MAGNESS, *Physiologist*, and H. C. DIEHL, *Junior Physiologist*, Office of Horticultural Investigations, Bureau of Plant Industry, United States Department of Agriculture

The successful holding of apples in storage is of great importance in this country, not only from the point of view of the commercial fruit industry, but from the viewpoint of the consuming public as well. Fresh fruit is assuming a position of increasing importance in the diet of the human race. The marketing of the fall and winter varieties of apples direct from the tree is practically limited to three months of the year. If a supply of this fruit is to be available throughout the year, storage suitable for holding the fruit is essential. Not only does proper storage insure a supply of a necessary food material throughout the year, but it also greatly stimulates consumption and stabilizes prices for the fruit grower.

Although much study has been devoted to the question of proper storage conditions for apples and other fruit, the problems involved are so complex that many questions remain unanswered. The factors of the exact influence of time of picking, storage temperature, humidity, ventilation, etc., upon the physiological ripening processes in the fruit are not perfectly understood, and many inaccurate impressions exist in regard to their functions in storage management.

In the studies here reported, the literature concerning the chemistry and physiology of the ripening processes in apples have been freely drawn upon and correlated with the investigations on the storage life of the fruit made by the authors.

### THE RIPENING OF APPLES ON THE TREE

For a proper understanding of the changes that occur in apples while in storage, it is important that the processes of growth and development of the fruit while on the tree be considered. The physiological changes in the apple fruit from the time of blossoming until ultimately consumed or until the end of its life is reached are continuous. The conditions under which the fruit is grown and the time of picking have an important bearing upon the storage behavior of the fruit.

### PHYSICAL CHANGES DURING DEVELOPMENT OF THE FRUIT ON THE TREE

Among the most important of the physical changes in apples during development and ripening on the tree may be mentioned increase in size, changes in color, changes in the skin texture, and changes in hardness of the flesh. These changes are all intimately associated with storage quality in apples.

<sup>1</sup> Accepted for publication Nov. 19, 1923.

<sup>2</sup> This paper gives the results of a portion of the work carried on under the project "Factors Affecting the Storage Life of Fruits."

## INCREASE IN SIZE OF THE FRUIT

The data on increase in size of apples, particularly during the latter part of the growing season, are extremely meager. Whitehouse (27)<sup>3</sup> measured the longitudinal and transverse diameters of Grimes apples at intervals, and found a steady increase in size from early June until September 20, when the last records were made. Actual diameter increase was somewhat less during the late season, but volume increase was very rapid. Curves based on Whitehouse's data are shown in Figure 1. In Figure 2 are shown measurements on diameters of 50 Winesap apples at Arlington, Va., during the summer of 1919.<sup>4</sup> Since the volume of the fruit increases approximately as the cube of the diameter, the second curve has been calculated to show the comparative increase in volume.

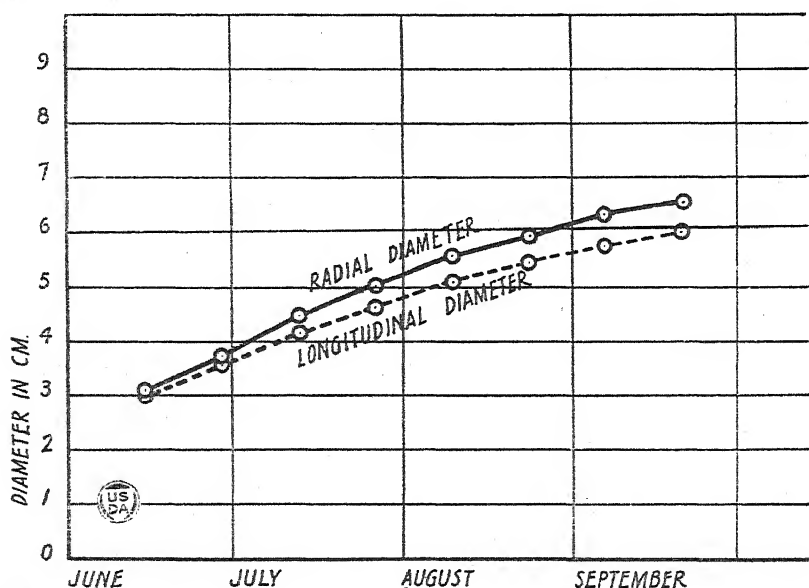


FIG. 1.—Increase in diameter of Grimes apples, Corvallis, Oreg., in 1915, after data by Whitehouse.

The season of 1919 at Arlington had a very heavy rainfall in July, somewhat less than normal in August, less than half of normal in September, and a heavy rain on October 1. It will be noted that there was a rapid increase in size from July until August 21, with only slow growth until September 19. A very rapid growth occurred between September 19 and October 10, most of which probably occurred after the heavy rain of October 1. No frost occurred in October.

These data indicate that if there is moisture available a large increase in size may occur very late in the growing season. There is much need of further investigations to determine how late this growth will continue under various conditions. Quantity of fruit on the tree as well as soil moisture will markedly influence the rate of increase in size. The size

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 36-38.

<sup>4</sup> The authors are indebted to L. A. Hawkins, physiologist, and L. B. Scott, pomologist, Office of Horticultural Investigations, for these data.

that the fruit has attained will often be an important consideration in determining when the fruit should be picked.

Increase in size of the fruit, at least during the latter part of the growing season, appears to be due largely to increase in size of the individual cells rather than to increase in their number. Consequently, fruit which attains large size will be composed of larger cells, and will be less firm and compact in texture than small or medium sized apples of the same variety. Medium or small sized fruit with compact firm texture is almost invariably superior for storage purposes to large size fruits, but the

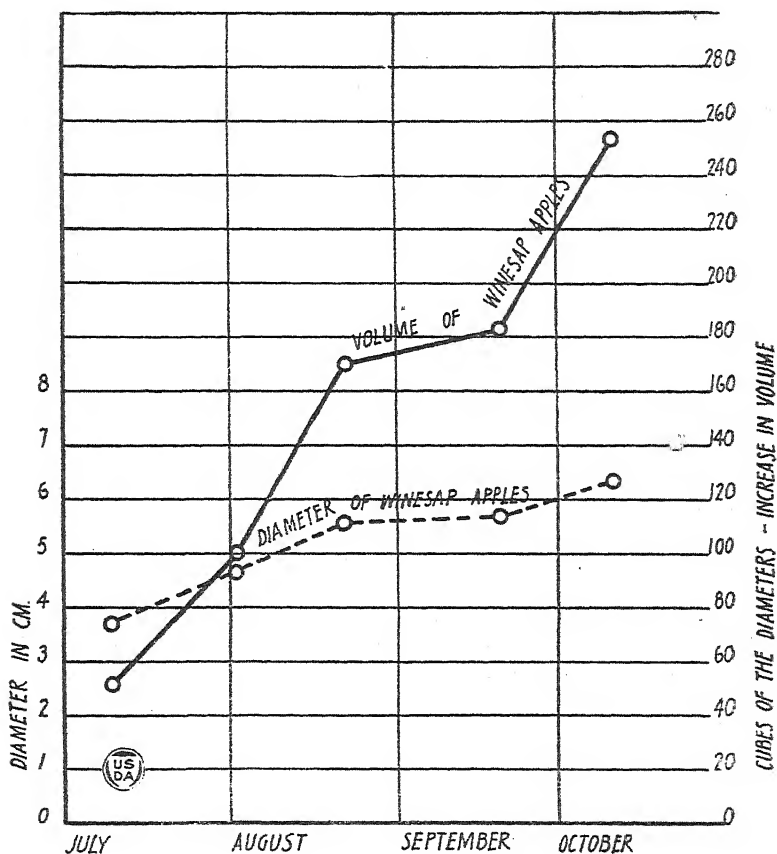


FIG. 2.—Increase in diameter and in volume of Winesap apples at Arlington, Va., 1919.

market usually pays a premium for large size in apples. Cummings and Lombard (9) have pointed out an apparent association between size of cells and storage quality in apples.

#### CHANGES IN COLOR ASSOCIATED WITH RIPENING ON THE TREE

The changes in color as the fruit ripens on the tree are twofold. There is a gradual increase in the red pigment developed in the subepidermal cells, the color increasing both in area and in intensity as the season advances. At the same time, the green or ground color gradually gives way to greenish yellow and finally to yellow.

The chemical changes which are responsible for color development in the apple have not, so far as the writers are aware, been determined. The cells in which the pigment is located are primarily subepidermal. There is no pigment in the cuticle, or wax covering, and no color in the epidermis, or outer cell layer. Below the epidermis, however, chlorophyll pigment, and later the red pigment, is very abundant. These pigments are largely limited to the rather flat, thick-walled cells lying in the immediate 2 to 10 layers beneath the epidermis, though in certain varieties some pigment, both green and red, occurs well down into the flesh of the apple.

As the red pigment develops it apparently replaces the chlorophyll, being developed in the same cells in which chlorophyll was formerly present. If red color does not develop, the disappearance of the chlorophyll leaves the yellow color characteristic of ripe and mellow fruit. The amount of red which develops is apparently directly associated with the exposure of the fruit to the light. The side of the fruit exposed to the direct rays of the sun is always more highly colored than the side away from the sun. Those regions of the country which have the greatest amount of sunshine during the late growing season produce the most highly colored apples. Treatments such as cultivation, or fertilizing with nitrogenous materials, and which result in a more dense foliage on the trees, will invariably result in more poorly colored fruit. On the other hand, sod culture, summer pruning, etc., which result in a more direct exposure of the fruit to the light, produce a more highly colored fruit. Shading will almost entirely prevent the development of red color. Whitehouse (27) has estimated color development of Fameuse and Tompkins King, and concludes that the greatest color development occurs during the last few days of the season. This would seem particularly to hold true after some of the leaves had fallen, thus exposing the fruit more directly to the sun. Since color development is largely dependent upon direct sunlight, picking the fruit and removing it from the sun will stop the development of the red pigment.

The change of the green or ground color to yellow, which occurs in most blushed varieties, is entirely independent of light. This change occurs regardless of whether or not the fruit is separated from the tree, and is one of the best indexes to the actual condition of maturity of the fruit. Corbett (8) has discussed this color change as a means of determining the time of picking the fruit. Although there is some variation in different varieties, practically all blushed apples should not be picked before they show a distinct yellowish tinge to the green of the unblushed side. This test, being largely independent of light exposure of the fruit, is very valuable as a picking aid.

#### CHANGES IN THE SKIN OF APPLES DURING RIPENING

The changes in the skin of the apple during its development on the tree have been studied in detail by Zschokke (28). He found the skins of young apples 4 to 5 weeks old to contain 2 to 10 stomata per square millimeter. No further stomata were formed, and as the fruit increased in size the stomata became farther and farther dispersed. During the latter part of the growing season cork formation usually occurs below the stomata openings. The openings are later ruptured by the growth of the fruit. The corky tissue with the ruptured stomata constitute the "dots" by which many varieties are characterized. In some varieties, however,



Zschokke found the stomata ruptured before the cork cells had formed, in which case protection was provided by the drying down of the underlying tissue. This, however, afforded opportunity for the entrance of microorganisms.

The epidermal covering of the apple consists of a cuticle, or wax coating on the outside, a layer of thick-walled epidermal cells, and below this a subepidermal region, consisting of a number of layers of thick-walled small and compact cells. The cells of the subepidermal region gradually become larger as the distance from the epidermis increases. The pigment, both red and green, is contained primarily in these subepidermal cells.

There apparently is a progressive thickening, not only of the cuticle, but of the subepidermal area as well, as the season progresses. Zschokke found a distinct variation in the thickness of these regions with the exposure of the fruit to the sun as well as with the variety. The writers have noted a distinct variation in the thickness of the subepidermal region upon the blushed and the unblushed sides of the same fruit, heavily blushed sides of Rome Beauty showing 8 to 10 cell layers in this region, whereas the unblushed sides showed about 6 layers.

The storage quality of fruits is undoubtedly closely associated with skin condition. Not only is the skin condition important in determining the amount of wilting in storage but it is of primary importance in determining the resistance of the fruit to decay. The development of storage scald and other physiological troubles is also closely associated with skin condition. Cummings and Lombard (9), in a limited study, found an apparent association between thickness of the cuticular layer as a variety characteristic and keeping quality in apples. Perry and Martin (23) report a similar correlation. Practical fruit handlers have long judged the keeping quality of apples partially by the amount of wax present on them. There is much need for a more exact study of the effect of climatic factors upon the epidermal covering of fruits in order to judge more accurately the effect of these conditions upon the storage life of the fruit. The skin of the fruit as a whole is extremely important, not only as a mechanical protection for the tissues beneath, but also as a partial regulator of the actual physiological processes going on in the fruit. This latter is accomplished through the action of the skin in limiting gaseous exchange.

#### THE SOFTENING OF APPLES ON THE TREE

There is probably no test so universally employed to determine the maturity of fruit as the resistance of the flesh of the fruit to pressure as estimated by pressing the fruit with the thumb. Lewis, Murneek, and Cate (18) used a mechanical tester to measure the rate of softening of pears on the tree. They found the fruit to soften rather rapidly, resistance to pressure decreasing at a rate of approximately 2 per cent per day.

Apples are usually removed from the tree when in a "hard ripe" condition. Color, ease of separation from the tree, amount of dropping, color of seeds, etc., as well as the softness of the fruit, are used to determine the time of picking. Notwithstanding the fact that apples are usually picked when "hard ripe," there is in most varieties a very considerable softening of the fruit before its removal from the tree. The rate of this softening has been determined by means of a mechanical pressure tester similar in design to that described by Murneek (21).

## Measuring the Softening

From 25 to 40 apples, selected to represent the averages of the trees from which they were taken, were used for each determination of

the hardness of the fruit. Apples from the same trees were used in successive tests, made at intervals of from 5 to 7 days.

Three tests were made upon the exposed flesh of each apple. The skin was removed from an area of about three-fourths of an inch diameter at three points equidistant about the circumference of the fruit, and the test made of the number of pounds pressure required to force a smoothly rounded plunger seven-sixteenths of an inch in diameter into the flesh of the fruit for a distance of five-sixteenths of an inch. Tests were also made of the same apples with skin intact, but it has been found that tests directly on the flesh of the apples are a more accurate index to the real condition of the fruit.

Unfortunately, these tests were not begun until rather late in the season, when softening may have been considerably advanced. When initial tests were taken, however, the fruit was not well colored, and lacked very appreciably of having attained its final size. Exact growth measurements were not made.

Figure 3 shows in graphical form the resistance to pressure of Delicious, Rome Beauty, Winesap, and Ben Davis apples. At the time

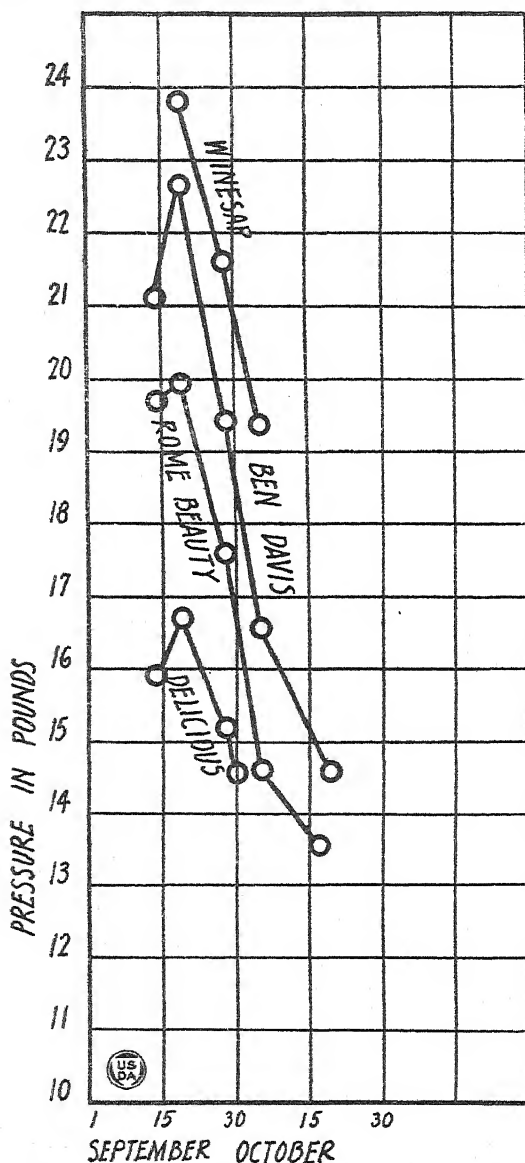


FIG. 3.—Softening of Winesap, Ben Davis, Rome Beauty, and Delicious apples while on the trees, Arlington, Va., 1922.

of making the initial pickings of Delicious, Rome Beauty, and Ben Davis the pressure-testing apparatus was not ready, and the fruit was held at 32° F. for

one week before testing. Initial tests on these varieties were lower than those following, probably because of this treatment. Under conditions prevailing during these tests, Ben Davis, Winesap, and Rome Beauty all softened very rapidly, and Delicious somewhat more slowly. The month of September, 1922, was very warm and dry at Arlington, Va., and October was practically without precipitation and moderately warm. Burroughs (5) found that Wagener apples growing on young trees in northern Pennsylvania softened only very slowly during September and October. These trees had received nitrate fertilizer and were very vigorous. It is not known whether the rate of softening while on the tree is primarily a variety characteristic or whether it varies largely with environmental factors.

In general, the harder the fruit is at time of picking and placing in storage the firmer it will be when removed from storage and exposed to market conditions. A firm, hard fruit, sufficiently well colored to be attractive, and sufficiently well ripened to be of high quality, and to escape storage scald, is the ideal for storage. High quality and skin condition associated with resistance to storage scald can be obtained only at the expense of a softening of the fruit. There is much need for a more exact knowledge of the influence of such factors as temperature, soil moisture, fertilizers, age and vigor of the trees, size of crop, etc., upon the rate of softening of the fruit. It is highly desirable that the fruit when picked be as firm as it is possible to secure it.

#### CHEMICAL CHANGES IN APPLES ASSOCIATED WITH RIPENING ON THE TREE

Numerous analyses have been made of the chemical composition of apples and other fruits at various times during their development on the tree. While the actual percentages of the various constituents will vary widely, depending upon the variety, locality, cultural conditions, and many other factors, the general changes have been well established and will be briefly reviewed. These changes may be considered under the following subjects: (1) Changes in total acidity, (2) changes in the sugar and starch content, (3) changes in the pectin content, and (4) changes in the moisture content of the fruit.

##### CHANGES IN TOTAL ACIDITY ASSOCIATED WITH RIPENING ON THE TREE

For more than a century, acidity changes in fruits have attracted wide attention, and many conflicting opinions have been held concerning the source of fruit acids, as well as their disappearance. This literature has been thoroughly reviewed by Bigelow, Gore, and Howard (2). Because of the limited chemical knowledge and the widely varying conditions under which early writers on this subject worked, many of their results are untrustworthy. Only the more recent contributions will be discussed here.

Browne (4) analyzed Baldwin apples and found a marked decrease in acidity at intervals from August 7 until December 15, when recorded as percentage of the wet weight of the apple. Gerber (12) reported a similar decrease in percentage composition, but pointed out the fact that the total amount of acid per apple increases rather than decreases as the season advances, the apparent decrease being more than counteracted by the increase in size of the fruit. Finally Bigelow, Gore, and Howard (2) in a very complete series of analyses on both summer and winter varieties of apples, found the percentage of acid to drop very rapidly from June until

September in all varieties. From the middle of September until the first of November the percentage of acid remained practically constant in the winter variety group which they analyzed (Winter Paradise, Huntsman, and Ben Davis). When the actual quantity of acid in grams per apple was calculated, however, these authors found that the amount of acid per apple increased until August, fell off very slightly during September, and again increased during October. It is probable that the decrease during September was due to local seasonal conditions.

In Figure 4 the changes in acidity for Ben Davis, Delicious, and Rome Beauty grown at Arlington, Va., during the ripening season of 1922 are shown graphically. It is apparent that from September 14 until mid-October there was a constant, though rather slight, decrease in the total acidity on the basis of percentage composition. Unfortunately, data on the relative size of the fruit at the time different determinations were made are not available. The fact, however, that there was a marked increase in size, particularly in Rome Beauty and Ben Davis, between September 14 and October 18 strongly indicates that the actual acidity in grams per apple increased rather than decreased during this period.

To summarize, then, the changes in acidity occurring while the fruit is on the tree, there is a marked decrease in percentage of acid in the fruit during most of the growing season, this decrease becoming less pronounced as the fruit approaches maturity on the tree. This decrease in acidity does not involve an actual disappearance of acid from the fruit, as the increase in size of the fruit is sufficient to simply dilute the acid already there. The most accurate conception of the acidity changes in apples while on the trees apparently is that of high acidity early in the

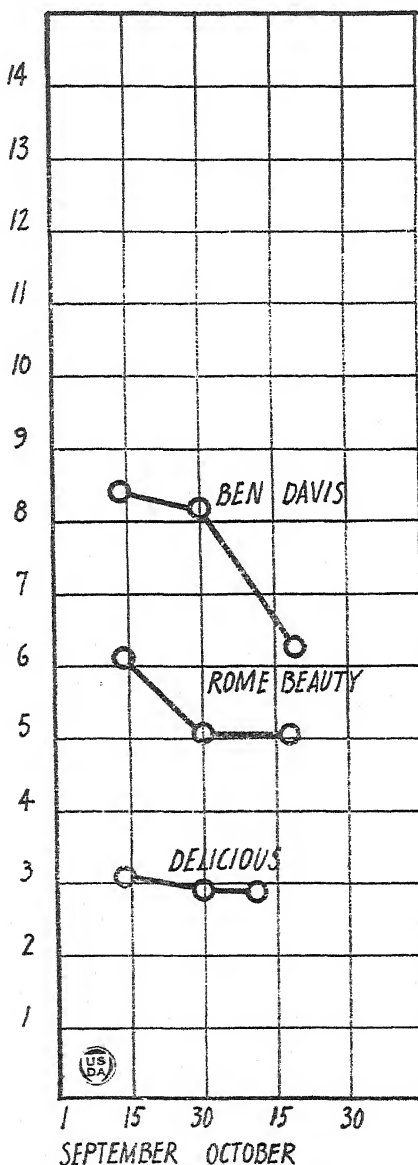


FIG. 4.—Decrease in acidity in apples while remaining attached to tree, Arlington, Va., 1922. Acid as cc. N/10 per 10 gm. wet tissue.

season, followed by dilution of the acid as the fruit increases in size, with consequent lower acid concentration in the mature fruit.

The amount of acid which the fruit of any variety may contain will vary widely with the conditions under which the fruit is grown. Con-

siderable variation occurs in individual apples from the same tree, depending upon the nutrition of the individual apples. Ballard, Magness, and Hawkins (1) have reported that apples from branches of Yellow Newtown trees which were girdled in June contained distinctly more acid, as well as more total sugar, than did apples from normal branches of the same trees, while fruit from partially defoliated branches on the same trees showed still less acid than the untreated checks.

In other works by the same investigators (unpublished) acidity determinations were made on fruit from a large number of Yellow Newtown trees during each of three succeeding seasons. These trees were about 20 years of age, and during the first and third seasons produced only a very light crop, averaging 1 to 6 bushels per tree. During the second season under test, however, the crop was very heavy. It was found that the average acidity in fruit from the same trees was fully 25 per cent higher during the "off" or light crop years than during the season of heavy production. These figures represent an average of about 70 individual trees. Furthermore, during the "off" year acidity was much lower on the average in the trees bearing a fairly full crop.

It is apparent, then, that there is a close association between acidity in apples and the nutritive conditions in the tree upon which they were produced. Climatic conditions also are of undoubted importance, although they have not been carefully studied. Acidity is of much importance in determining quality in fruit not only as relates to growing conditions, but as regards storage as well, for it is intimately associated with flavor.

#### CHANGES IN WATER CONTENT OF THE FRUIT

The change in water content of the fruit as it matures on the tree is less marked than changes in sugars. The percentage of water in the fruit will vary widely with variation in soil moisture. Conditions of soil moisture being uniform throughout the season, however, there is a slight decrease in the amount of water and an increase in dry matter as the season progresses. This change is not marked, but is borne out by the results obtained by Bigelow, Gore, and Howard (2), by Burroughs (5), and by other investigators.

#### CHANGES IN SUGARS AND STARCH DURING GROWTH

The literature regarding changes in sugar in fruit during its ripening on the tree is equally as voluminous as that regarding acidity changes. The following summary is based on the work of Bigelow, Gore, and Howard (2) which included a careful study of the sugar changes in apples both while attached to the tree and while held in storage following picking.

During early summer (June) the total sugar content of all the winter varieties was low, running from 3 to 4 per cent of the weight of the green tissue. In both summer and winter varieties, the percentage of total sugar increased steadily until the fruit was dropping badly, that being late July for the summer varieties (Early Strawberry, Bough, and Yellow Transparent) under West Virginia conditions, and late October for the winter varieties (Ben Davis, Huntsman, and Winter Paradise). During the early season, the sugar is largely in the form of reducing sugars, but as the season advances there is a marked increase in the

amount of sucrose present, as well as an increase in reducing sugar. In some varieties at the end of the growing season the quantity of reducing sugar and of sucrose was approximately equal, while in others the reducing sugar was approximately double the sucrose in quantity.

The starch content of the fruit, in terms of per cent of the green weight, reached its highest point in the summer varieties in June and in the winter varieties in July. The actual total quantity of the starch per apple, however, did not reach its maximum until much later, about mid August, in the winter varieties. From this time forward there was a decrease not only in percentage composition of starch but in actual starch per apple, indicating that there was an actual starch hydrolysis going on after this date. With starch hydrolysis there is a sharp increase in sucrose, indicating that the sugar storage form which replaces the starch storage as the fruit matures is primarily sucrose.

#### CHANGES IN THE PECTIN MATERIALS

There is much confusion regarding the changes that occur in the pectin materials during the ripening processes of fruits. The voluminous work on pectins has been admirably reviewed by Bigelow, Gore, and Howard (2) and need not be repeated here. Because of the probable intimate association of changes in the pectin materials with the very important softening of the fruit in storage, however, a brief summary of these changes is desirable.

It is apparent that there is a water-insoluble pectin compound which acts as a cementing material in green fruit, as well as in other fleshy plant tissue. The exact chemical nature of this substance is not established, and it has been mentioned in the literature under various names, as pectose, pecto-cellulose, and calcium pectate. The more recent work indicates that this substance is calcium pectate. This calcium pectate cements the cell walls of the green fruit flesh firmly together, and is largely responsible for the "hardness" of green fruit, since such fruits can be crushed only by actually breaking the cells.

As the fruit ripens, this cementing material slowly hydrolyzes, probably by enzymatic action, into substances more readily soluble in water. This material is that generally referred to under the name "pectin," and its formation, which occurs slowly under natural conditions, may be greatly hastened by boiling with water. Pectin, according to some investigators, can be further split by hydrolysis, either with boiling water or by boiling with acids or alkalis. With alkalis, pectic acid is first formed, followed by meta-pectic acid after prolonged boiling. Meta-pectin, a closely similar substance, has also been formed. The products of final hydrolysis have usually been pentose sugars and galactose. More recently Schryver and Haynes (25) and Ehrlich (11) have worked upon the chemical nature of pectins. The former reported that the pectin obtained from a number of fruits was essentially identical and found the molecule to contain pentose sugars sufficient to account for about one-third its weight. Ehrlich reports pure pectin as consisting primarily of arabinose, galactose, and galacturonic acid.

The changes in the pectins as they occur naturally in the fruits have not been worked out in association with the physical changes in the fruit. The fact seems well established that the cementing material in green fruits is calcium pectate, and that this material accounts for the hardness

of the fruit, since it results in the cells being ruptured when pressure is applied, rather than simply separating. As the cementing material is hydrolized, the cell walls tend to separate more and more readily and the fruit becomes progressively softer. This fact also probably explains the juiciness of green apples, and the apparent lack of juice in the soft or mellow fruit. The juice is primarily within the cells, and escapes only when the cell walls are broken. If the cells can separate readily, the individual cell walls will not be broken to an appreciable extent when the fruit is crushed. Consequently the soft or "mealy" fruit also appears "dry," although actually containing practically as much moisture as when hard. This apparent dryness may also be caused in part by the greater absorption of water by the cell colloids as the fruit softens.

As the fruit ripens, both on the tree and in storage, these changes in pectin materials go on. It has not been established how far they progress, but it is often asserted in the literature that the "pectin decreases as the fruit ripens." This usually has referred to pectin extracted by boiling water, and would include both "pectin" and calcium pectate or "pectose." This would indicate that as the fruit ripens and softens hydrolysis may go so far that some of the pectin materials will break down to sugars. The sugars of the fruit may thus be very slowly augmented throughout the life of the apple. Because of the complexity of the compounds involved and the large quantities of various sugars always present in fruit tissue, these points are very difficult to establish.

#### SUMMARY OF CHANGES IN THE FRUIT WHILE ON THE TREE

The changes associated with growth and ripening may then be summarized as follows: There is a continuous increase in size of the fruit from blossoming time until late season. This increase will vary greatly with growing conditions. Growth in size is accompanied by changes in the skin, the wax being formed during the latter part of the growing season, and the lenticels being corked over. Associated with these changes are color changes. Red pigment may develop just below the epidermis on fruits exposed to sunlight. The green color gradually gives way to yellowish green or yellow as the chlorophyll disappears. The fruit softens as it ripens, the rate of softening also undoubtedly varying with growing conditions. Associated with all these physical changes, there is a gradual decrease in acidity and an increase in sugars as the fruit ripens. Starch during the late growing season gradually changes over to sugar. Changes in the pectin constituent of the cell walls, consisting probably in insoluble calcium pectate being changed to water-soluble pectin or pectic acid, allows the cells to separate and break more readily and results in a softening of the fruit.

#### CHANGES IN APPLES FOLLOWING THEIR REMOVAL FROM THE TREE

The removing of fruit from the tree entirely stops certain of the processes going on in the fruit, while others continue in much the same manner as though the fruit remained on the tree. Increase in size stops, as well as development of red color. The latter is probably due to the removal from direct exposure to light rather than to the cutting off of any substance derived from the tree. The green color of the unblushed surface continues to disappear, and the yellow color, previously masked by the green, becomes predominant. It is not known whether or not any changes in the epidermal covering occur following

picking. Softening, already under way while the fruit was on the tree, proceeds rapidly after removal from the tree, unless the fruit is placed at once in a low temperature.

The chemical changes in progress when the fruit is picked from the tree continue mainly in the same direction after picking, though the rate of the various changes may not be the same. From time of picking forward, there is a continuous drop in acidity. Starch present in the fruit quickly changes to sugar, as has been shown by the work of Bigelow, Gore, and Howard (2), of Otto (22), of Magness and Burroughs.<sup>6</sup> Following completion of this change, there is little further variation in total sugars, although there is undoubtedly a very small loss due to respiration. Even this loss may be partially replaced by acquisitions from the pectin materials. Bigelow, Gore, and Howard found that as the fruit was held in storage there was a continual decrease in the percentage of sucrose and a corresponding increase in reducing sugar. Pectin changes, associated with the softening of the fruit, continue throughout the storage season.

#### EFFECT OF STORAGE CONDITIONS UPON CHANGES IN FRUIT FOLLOWING PICKING

Among the conditions in storage houses that are within the control of the operator or fruit handler may be included the factors of temperature, humidity, air renewal, or ventilation, air stirring or movement within the room, and the type of package in which the fruit is packed. These factors have been studied in relation to their effect upon certain of the changes occurring in the fruit while in storage. The studies have consisted primarily in determining the effect of these different factors upon the rate of softening of the fruit, upon the acidity changes in the fruit, and upon the general metabolic activity as measured by respiratory activity. Shrinkage in weight, due to loss of moisture from the fruit and loss through respiratory activity, has also been studied.

#### EFFECT OF TEMPERATURE OF STORAGE UPON RATE OF SOFTENING

Magness and Burroughs<sup>6</sup> measured the rate of softening of eight varieties of apples under different storage conditions. Composite curves, from their data on Jonathan, Delicious, Rome Beauty, Winesap, Baldwin, York Imperial, Esopus (Spitzenberg), and Yellow Newtown, at 36° and 32° are reproduced in Figure 5. It will be noted that the rate of softening in storage was approximately exactly twice as rapid at 36° as at 32° F., fruit at 36° being as soft after two months in storage as fruit at 32° was at the end of four months.

Figures 6 to 11, inclusive, show the relative rates of softening of Delicious, Rome Beauty, Ben Davis, Rhode Island Greening, Winesap, and York Imperial, respectively, when held at 70° F. and at 32° F. Figures 6, 7, and 8, for the first three named varieties include data on fruit picked at three different intervals, approximately two weeks apart.

It is at once apparent that softening in all varieties was greatly accelerated by higher temperatures. However, it is also apparent that there is a distinct difference in the response of different varieties to the various temperatures.

<sup>6</sup> MAGNESS, J. R., and BURROUGHS, A. M. APPLE STORAGE INVESTIGATIONS. The Marble Laboratory, Inc. 2d Rept. Not yet published.

<sup>6</sup>MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.



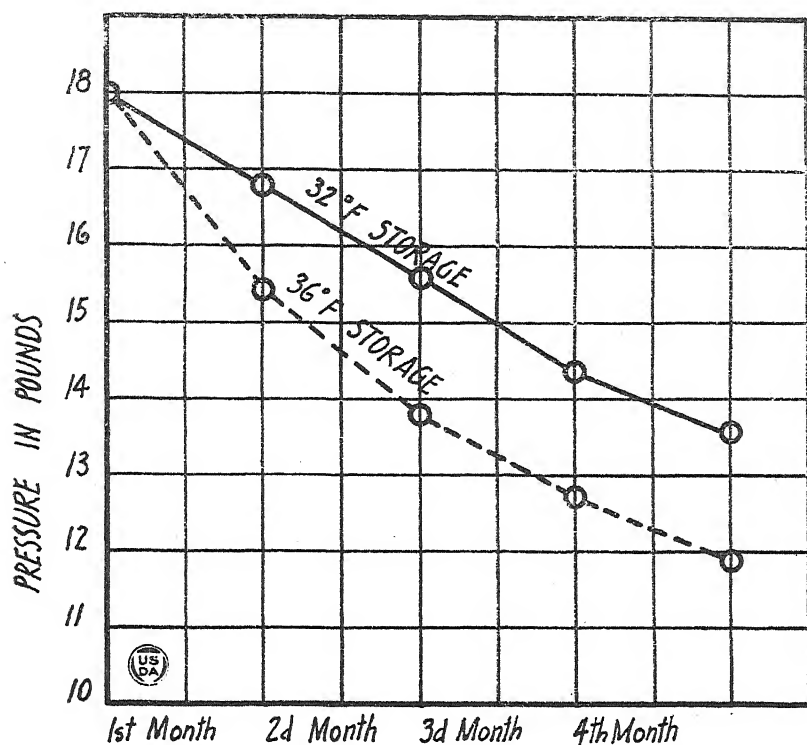


FIG. 5.—Softening rates of apples in storage. Composite curves of Jonathan, Delicious, Rome Beauty, Winesap, Baldwin, York Imperial, Esopus (Spitzenberg), and Yellow Newtown. After Magness and Burroughs.

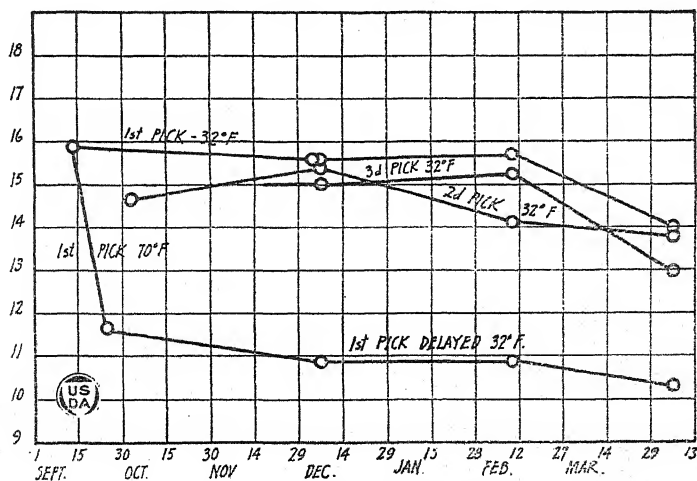


FIG. 6.—Softening of Delicious apples in storage.

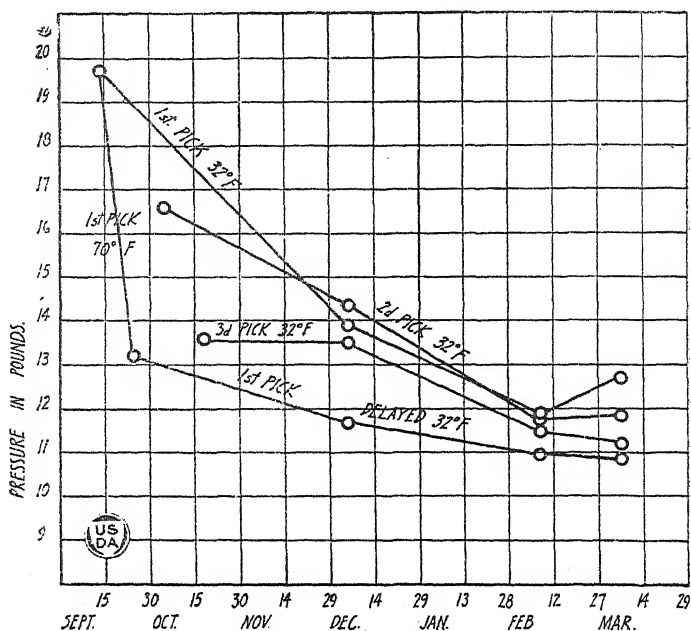


FIG. 7.—Softening of Rome Beauty apples in storage.

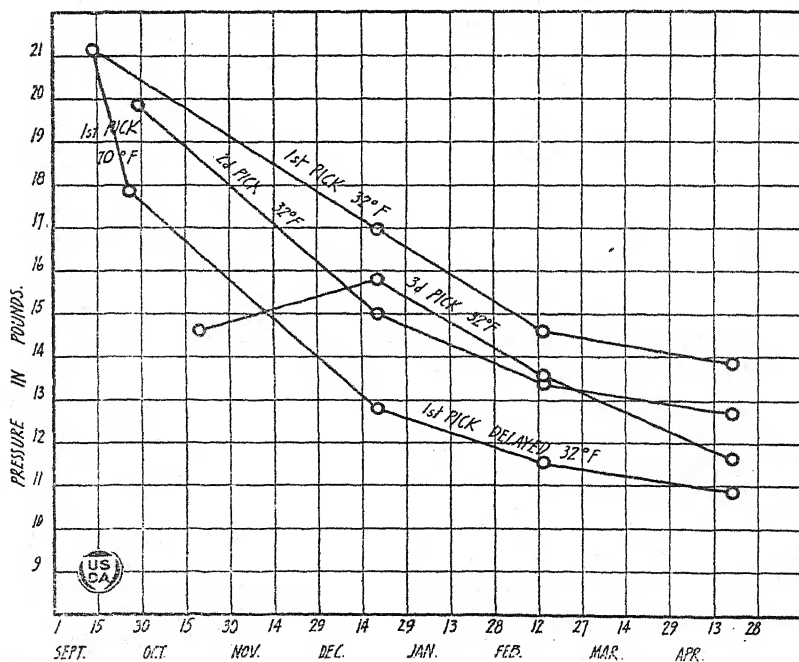


FIG. 8.—Softening of Ben Davis apples in storage.

Delicious and Rhode Island Greening (figs. 6 and 9) softened with great rapidity, and after 10 days at 70° were practically full soft. Both varieties were softer at the end of 12 days exposure to 70° than they were at

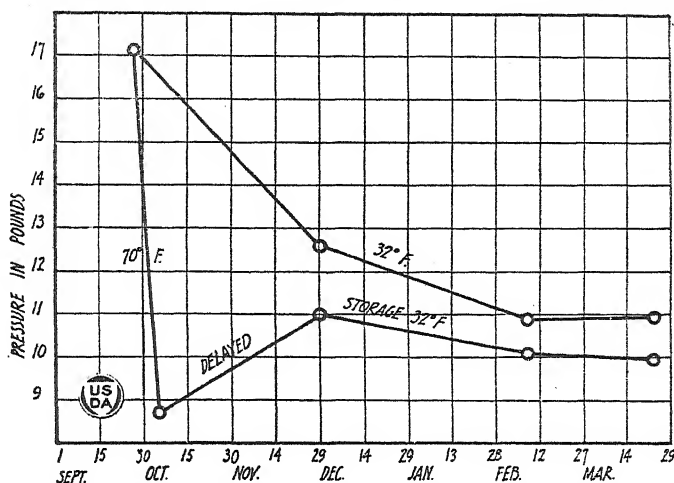


FIG. 9.—Softening of Rhode Island Greening apples in storage.

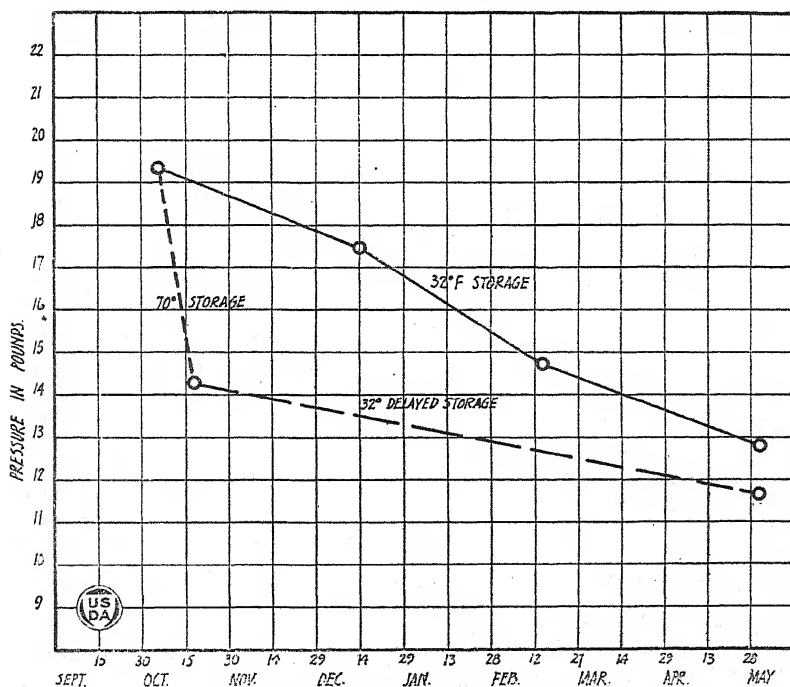


FIG. 10.—Softening of Winesap apples in storage.

the end of 6 months at a continuous temperature of 32°. With Delicious particularly, fruit placed directly in 32° storage softened very little, even after several months in storage. Rhode Island Greening softened more

rapidly in  $32^{\circ}$  temperature than did Delicious and also softened with great rapidity at  $70^{\circ}$ .

Rome Beauty and Winesap were somewhat harder when picked, and while the softening rate was rather rapid, the fruit was in much better condition after 10 to 12 days at  $70^{\circ}$  than were the Delicious and Rhode Island Greening apples.

Ben Davis of all pickings softened with unexpected rapidity in cold storage, and surprisingly slowly at  $70^{\circ}$  F. While Delicious and Rhode Island Greening were harder after 6 months at  $32^{\circ}$  than after 12 days at  $70^{\circ}$ , and Rome Beauty and Winesap were as soft after 12 days at  $70^{\circ}$  as after 3 to 4 months at  $32^{\circ}$  F., Ben Davis softened as much in  $2\frac{1}{2}$  months at  $32^{\circ}$  as in 12 days at  $70^{\circ}$ .

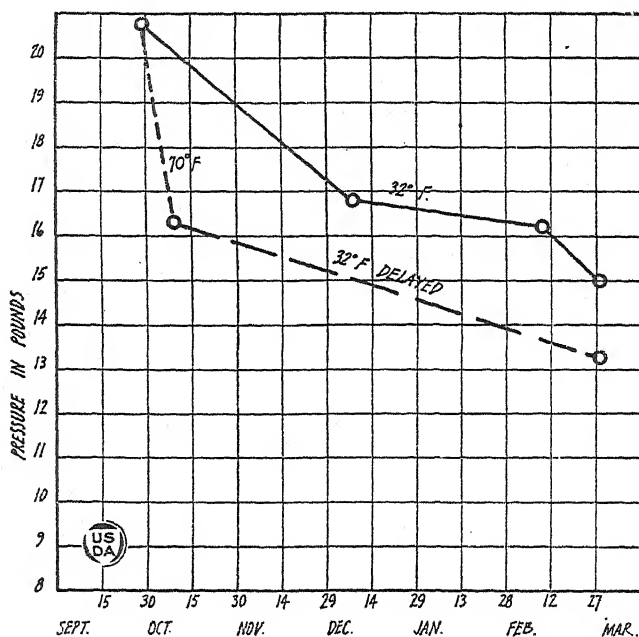


FIG. 11.—Softening of York Imperial apples in storage.

This indicates that the group of varieties which we class as "long keepers" are so, not primarily because they soften and ripen more slowly in cold storage than do the shorter keeping varieties, but because they soften less rapidly when out of cold storage. Thus it becomes particularly imperative that such varieties as Delicious and Rhode Island Greening be cooled quickly after picking, and be kept cold continuously if long storage is to be accomplished. Well colored Delicious can be held successfully until April if cooled promptly after picking. Such varieties as Ben Davis, Winesap, Yellow Newtown, and York Imperial soften relatively much less rapidly when exposed to higher temperatures, and consequently are less severely injured by delaying cold storage. All varieties, however, soften very much more rapidly at any temperature above  $32^{\circ}$  than at that temperature.

## EFFECT OF DELAYED STORAGE UPON HARDNESS OF THE FRUIT

A study of the data presented in Figures 6 to 11, inclusive, is interesting from the standpoint of the effect of delaying the time of storing after picking upon the keeping quality of the fruit. It is apparent that the fruit softens rapidly during this period and that throughout its storage life it remains much softer than fruit placed in storage immediately. Ramsey, et al. (24) have pointed out that apples so handled were usually softer, more yellow, and showed more decay when removed from storage than did similar fruit stored immediately. This is true for all varieties here studied, but is particularly marked for rapidly softening apples, such as Delicious, Rhode Island Greening, Jonathan, etc. These data are in full accord with the previous findings of Magness and Burroughs, who report that fruit in cellar storage in Pennsylvania (temperature range from 40° to 50° F.) reached full softness in from a month to six weeks' time.

These data all indicate clearly the importance of getting the fruit to cold storage as quickly as possible after picking if long holding is desired. Only when the fruit is cooled will the softening processes be checked. A temperature of 36° F. will apparently cause most varieties to soften approximately twice as rapidly as 32°, while 70° will cause softening to proceed from 5 to 15 times as rapidly as will 32°.

## EFFECT OF TYPE OF PACKAGE AND VENTILATION UPON RATE OF SOFTENING OF APPLES

Magness and Burroughs found no variation in the rate of softening of eight varieties of apples held in rooms at 32° F. continuously ventilated with outside air as compared with similar apples held in a room at the same temperature not ventilated but with good natural air movement within the room. Similarly, they found no variation in rate of softening in different packages when tests on the fruit with skin removed were compared. These tests included fruit stored in open crates, in wrapped boxes, and in barrels. Apparently because of wilting, fruit with skin intact was harder in the more open packages.

Tests have been repeated for the relative rates of softening of apples in different packages; with results similar to those reported by Magness and Burroughs. These results are summarized in Table I.

From the data in Table I it is apparent that there was no consistent difference in hardness of the fruit depending upon type of package. It should be stated that the oiled paper wraps used on the fruit in these tests were rather small in size, and that there was no appreciable transfer of oil from the wraps to the surface of the fruit. There is a widespread belief among commercial apple handlers that oil paper wraps result in slower ripening of the fruit. This will be further discussed under the subject of oil, wax, or paraffin coatings. It is apparent from the results here recorded, however, together with results previously reported by Magness and Burroughs, that so long as the surface of the fruit is not coated with any material which inhibits gaseous exchanges the rate of softening of the fruit under commercial storage conditions is not appreciably affected by ventilation, type of package, etc. Temperature apparently is the one controlling factor in determining the rate of softening of the fruit, if held under conditions of normal respiration.

TABLE I.—Effect of type of package upon the rate of softening of apples in 32° F. storage

Variety.	Package.	Average pressure test when stored.	Average pressure test at end of storage season.
		Pounds.	Pounds.
Delicious.....	Barrel.....	15. 20	13. 47
Do.....	Oil paper wrapped box.....	15. 20	14. 47
Do.....	Non-oil paper wrapped box.....	15. 20	13. 72
Rome Beauty.....	Barrel.....	18. 16	12. 23
Do.....	Oil paper wrapped box.....	18. 16	11. 70
Do.....	Non-oil paper wrapped box.....	18. 16	12. 42
Ben Davis.....	Barrel.....	20. 52	13. 63
Do.....	Oil paper wrapped box.....	20. 52	12. 95
Do.....	Non-oil paper wrapped box.....	20. 52	12. 27
York Imperial.....	Barrel.....	20. 75	14. 70
Do.....	Non-oil paper wrapped box.....	20. 75	14. 85
Rhode Island Greening.....	Barrel.....	17. 13	10. 99
Do.....	Oil paper wrapped box.....	17. 13	11. 07
Do.....	Non-oil paper wrapped box.....	17. 13	10. 79
Winesap.....	Barrel.....	19. 33	12. 88
Do.....	Non-oil paper wrapped box.....	19. 33	12. 74

## EFFECT OF TEMPERATURE AND TYPE OF STORAGE PACKAGE UPON ACIDITY

Acidity is of marked importance in determining the eating and cooking quality of fruits. For this reason changes in acidity while the fruit is in storage are of much importance in determining quality in stored fruit.

Gerber (12) reported that the acidity disappeared from apples with great rapidity when the apples were held at 30° C. (86° F.) and believed that it was consumed by the respiratory processes of the fruit. At 18° C. (64 2/5° F.) acidity disappeared more slowly, and at lower temperatures (0° C. or 32° F.) it remained practically constant. Other workers, using other fruits, however, have obtained somewhat different results. Hawkins and Magness (16) and Hawkins (15) on grapefruit, Diehl and Magness (10) on plums, and Magness (19) on Bartlett pears, all found that the acidity decreased when the fruits were held at low temperatures, but remained practically constant, or in the case of Bartlett pears and plums actually increased, when the fruit was stored at temperatures of about 60° F. Bigelow, Gore, and Howard (2) found a constant decrease in acidity when apples were removed from 32° storage and held at 60° F. or 70° F. Magness and Burroughs<sup>7</sup> found acidity to decrease most rapidly in apples stored in a cellar (40° to 50° F.), less rapidly when stored at 35° F., and still less rapidly when held at 32° F.

Figures 12 and 13 show changes in acidity in the six varieties of fruit here studied both during the time they were in storage at 32° F. and during an initial period of 12 days before certain lots were placed in storage.

Acidities were determined by cutting plugs of apple tissue with a cork borer, preparing these in a finely divided condition by passing through a sampling press of the type described by Clark (6), weighing out 100 grams, boiling and making up to 1,000 cubic centimeters volume with distilled water. Samples were preserved with toluol, and filtered and titrated after three days' extraction. For titration, N/10 NaOH

<sup>7</sup> MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.

was used with phenolphthalein as indicator. The results are expressed in cubic centimeters of N/10 acid per 10 grams of wet tissue.

There is a constant, but slow, decrease in acidity in all varieties during the storage season. Total decrease is very nearly the same for all varieties, regardless of total acidity present. Thus Delicious, with very low acidity at the start of the season, lost acid about as rapidly as Rhode

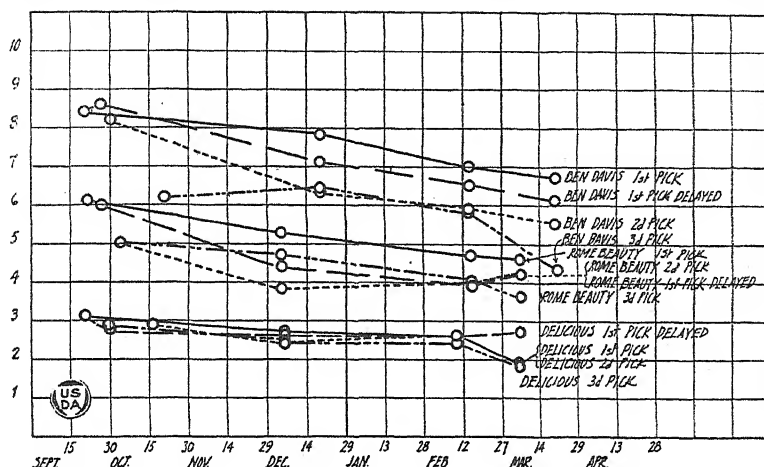


FIG. 12.—Acidity changes in Ben Davis, Rome Beauty, and Delicious apples while in storage. Acid as cc. N/10 in 10 gm. wet tissue.

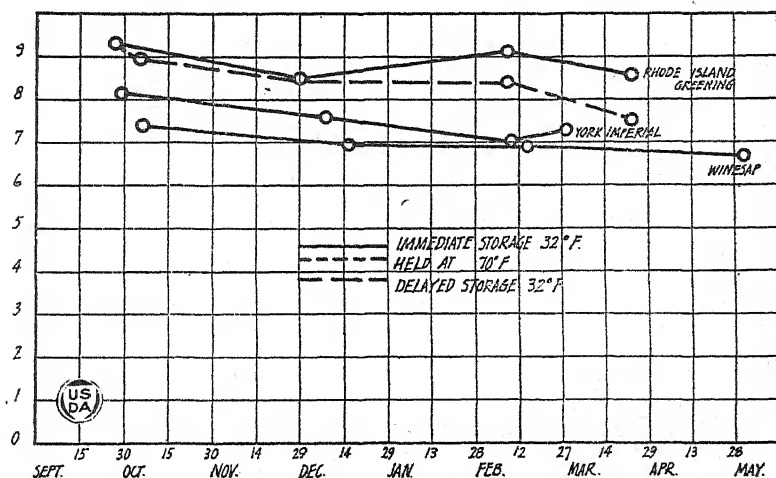


FIG. 13.—Acidity changes in Rhode Island Greening, York Imperial, and Winesap apples while in storage. Acid as cc. N/10 in 10 gm. wet tissue.

Island Greening, which was very high in acid content. Total loss in Delicious amounted to more than one-third the amount originally present, while loss in Rhode Island Greening was only about one-ninth the total.

Loss in acidity during an initial period in warm storage was relatively slight in all varieties, and in some varieties there was an apparent gain, due probably to variation in the samples. It is apparent, however, that

acidity decreased relatively less rapidly during exposure to warm temperatures than did softening of the fruit. It would seem from these data that fruit ripened rapidly at high temperatures would be higher in acidity for the same degree of softness than would the same fruit ripened more slowly at lower temperatures. Thus it appears that while apples will lose acidity when held at temperatures of around 70° F., this loss is less rapid in proportion to the other ripening processes than it is at 32° F.

It is also apparent from the data on which Figures 12 and 13 are based that acidity loss is relatively rapid in fruit remaining on the trees. Figures 6, 7, and 8 show that first-pick delayed-storage fruit was softer throughout the year than was second and third pick immediate-storage fruit. Figure 12 shows, however, that the early-pick delayed-storage fruit was distinctly more acid than the late-picked fruit. Rapid loss in acidity in fruit on the tree is undoubtedly due in part to the increase in size of the fruit.

#### EFFECT OF TYPE OF PACKAGE OR OF ROOM VENTILATION UPON ACIDITY OF THE FRUIT

Magness and Burroughs reported no consistent variation in acidity due to ventilation of the storage rooms, or to type of package in which the fruit was stored. Tests have been repeated on the effect of package on acidity of the fruit and are reported in Table II.

From the above data it is apparent that wide fluctuations exist in the acidity of individual apples or groups of apples, and that large numbers must be used if a figure representing the correct average is to be obtained. Individual figures in the above tables are based upon analysis of from 10 to 20 apples. The irregular variation in the data obtained indicates that this is not a sufficient number to represent accurately the condition of the various lots of fruit. It is apparent, however, that there is no distinctive variation in acidity due to type of package. Temperature, which regulates the rate of softening and general respiratory activity, appears also to regulate mainly the rate of acidity loss.

#### CHANGES IN COLOR OF FRUIT IN STORAGE

As discussed earlier, there appears to be no change in the red color of apples after they are removed from direct sunlight. The chlorophyll green tends to disappear in storage, however, leaving a yellow ground color. Careful observation indicates that this change in color occurs only very slowly in 32° F. temperature and rather rapidly at higher temperatures. It apparently is rather closely associated with the softening process in fruit exposed to normal storage conditions. Wide variation occurs, however, in the ground color of individual apples in storage which apparently can not be associated with the softness of the fruit.

#### CHANGES IN SUGARS, PECTINS, MOISTURE CONTENT, ETC., DURING STORAGE

Analysis to determine the chemical changes in the fruit here studied while in storage, other than change in acidity, have not been made. The work of Bigelow, Gore, and Howard (2), of Magness and Burroughs,<sup>8</sup> and of numerous other workers has shown that there is a sharp increase

<sup>8</sup> MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT



in sugars after picking, due to starch being transformed to sugar. Following this, there is probably a very slow dropping off in total sugar, due to respiratory activity. This decrease, however, is hardly sufficient to be detected by chemical analysis, because of the wide variation in individual fruits.

Pectin changes in apples while in storage have never been satisfactorily determined, because of the multiplicity of closely related compounds present and the difficulty of determining these compounds quantitatively. It is undoubtedly true, however, that as the fruit softens there is a gradual disappearance of the calcium pectate, or substance which acts as a cementing factor between cell walls.

TABLE II.—*Effect of type of package upon acidity in apples in cold storage—cubic centimeters N/10 acid per 10 grams of wet tissue*

Variety.	Type package.	Acidity beginning of storage season.	Acidity end of storage season.
Rome Beauty, first pick . . . . .	Barrel . . . . .	6.10	4.50
Do . . . . .	Wrapped box . . . . .	6.10	4.64
Rome Beauty, second pick . . . . .	Barrel . . . . .	5.05	4.85
Do . . . . .	Wrapped box . . . . .	5.05	4.28
Do . . . . .	Wrapped box, oil paper . . . . .	5.05	3.86
Ben Davis, first pick . . . . .	Barrel . . . . .	8.38	6.10
Do . . . . .	Wrapped box, oil paper . . . . .	8.38	7.28
Do . . . . .	Basket, unwrapped . . . . .	8.38	7.73
Ben Davis, second pick . . . . .	Barrel . . . . .	8.18	5.88
Do . . . . .	Wrapped box . . . . .	8.18	4.95
Do . . . . .	Wrapped box, oil paper . . . . .	8.18	5.61
Do . . . . .	Basket, unwrapped . . . . .	8.18	5.28
Delicious, first pick . . . . .	Barrel . . . . .	3.13	2.27
Do . . . . .	Wrapped box . . . . .	3.13	1.82
Do . . . . .	Wrapped box, oil paper . . . . .	3.13	1.68
Delicious, second pick . . . . .	Barrel . . . . .	2.90	2.01
Do . . . . .	Wrapped box . . . . .	2.90	1.64
Do . . . . .	Wrapped box, oil paper . . . . .	2.90	2.10
Rhode Island Greening, first pick . . . . .	Barrel . . . . .	9.31	7.75
Do . . . . .	Wrapped box . . . . .	9.31	8.55
Do . . . . .	Wrapped box, oil paper . . . . .	9.31	8.33

Changes in moisture content will vary directly with the storage conditions. If apples are held under conditions of high humidity, there will be practically no decrease in moisture, and there may be an actual increase, due to the water formed during respiration. On the other hand, if storage is in a very dry atmosphere, the loss of moisture may amount to several per cent during a relatively short storage season. Loss of moisture from the fruit also will vary greatly with the variety and the condition of maturity when picked.

#### LOSS OF MOISTURE AS RELATED TO PACKAGE IN WHICH APPLES ARE STORED

At the time the fruit was placed in storage certain average lots in each package were accurately weighed. These same lots were weighed again at the end of the storage season and the total shrinkage in weight was determined. Humidity in the room ranged from 85 to 90 per cent

of saturation with slow natural air movement. Humidity was sufficiently high so that traces of mold growth were present on the ends of boxes and inside the barrels. Under these conditions there was no shriveling discernible in any fruit, even after a long storage season in open boxes. Losses in weight are summarized in Table III.

TABLE III.—*Effect of type of storage package upon fruit shrinkage*

Variety.	Length of time in storage.	Type of package.	Shrinkage; loss in weight.
	<i>Months.</i>		<i>Per cent.</i>
Winesap.....	7	Barrels, closed.....	0.6
Do.....	7	Barrel, open.....	2.0
Do.....	7	Wrapped box.....	1.7
Do.....	7	Open box, fruits paraffin coated.....	1.1
Rome Beauty.....	5	Barrel, closed.....	.55
Do.....	5	Barrel, open.....	.8
Do.....	5	Wrapped box.....	.7
Do.....	5	Wrapped box, oil paper.....	.7
Do.....	5	Unwrapped box, fruits paraffin coated..	.3
Ben Davis.....	6½	Barrel, closed.....	.95
Do.....	6½	Barrel, open.....	1.15
Do.....	6½	Wrapped box.....	1.5
Do.....	6½	Wrapped box, oil paper.....	1.4
Do.....	6½	Bushel basket, unwrapped.....	2.0
Do.....	6½	Unwrapped box, fruits paraffin coated..	1.0
Delicious.....	6½	Barrel, closed.....	.65
Do.....	6½	Barrel, open.....	1.25
Do.....	6½	Wrapped box.....	1.00
Do.....	6½	Wrapped box, oiled paper.....	1.00
Do.....	6½	Bushel basket, unwrapped.....	2.00
Do.....	6½	Unwrapped box, fruits paraffin coated..	.65
* Rhode Island Greening...	6	Barrel, closed.....	1.0
Do.....	6	Wrapped box.....	2.3
Do.....	6	Wrapped box, oiled paper.....	2.0

The data recorded in Table III are of primary interest in showing that if the storage room is kept sufficiently moist very little loss in weight will occur regardless of the type of package used. The storage season varied from five months for Rome Beauty to seven months for Winesap, yet the maximum loss in weight in open packages was only slightly over 2 per cent. This includes both moisture from the fruit and gaseous-respiratory products. Based upon the results of respiration tests, the loss in weight of apples due to respiration during six months at 32° F. would be from 0.2 to 0.3 per cent of the total weight. Hence all loss above 0.3 per cent may be attributed to moisture loss from the fruit. Part of this moisture may, however, be that formed by the respiration of the fruit.

Maximum losses in all varieties occurred in the most open containers. Closed barrels showed losses of less than 1 per cent, wrapped boxes somewhat more, and unwrapped baskets still higher shrinkage. Fruit coated thinly with paraffin showed very slight shrinkage, even in open containers.

While the losses in weight here reported were all slight, that will be true only when the room as a whole is kept humid. If the air becomes dry, heavy shrinkage will result, particularly in fruit held in open containers.

If fruit is stored in closed barrels, the humidity of the storage room is not a very important factor. In open barrels or boxes, however, it is important to keep the humidity up to at least 85 per cent, to avoid shriveling the fruit. In more open containers, as baskets or slatted crates, it is very essential that humidity be kept sufficiently high.

#### RELATION OF TIME OF PICKING TO LOSS IN WEIGHT

Tests of the rate of loss of weight in storage as related to time of picking were made upon the three varieties, Ben Davis, Rome Beauty, and Delicious. There was no distinctive variation in loss of weight that could be associated with time of picking with the picking dates here used, namely, September 14 to October 10. It is a well known fact that fruit picked when extremely immature will wilt more readily than will well ripened fruit, but apparently the skin of the apples here studied was sufficiently cutinized by September 14 to prevent excessive moisture loss.

#### EFFECT OF COATING THE SURFACE OF THE FRUIT WITH OIL OR PARAFFIN UPON THE RIPENING PROCESSES OF THE FRUIT

During the normal respiration of fruit, oxygen is taken up from the air, and carbon dioxide given off from the fruit. The carbon dioxide is generated throughout the tissue, penetrates to the epidermis, probably largely through the intercellular spaces, which are relatively large in apple tissue, and passes out through the epidermis, while oxygen enters by a similar route. Consequently, the condition of the epidermis is of great importance in determining the concentrations of  $\text{CO}_2$  and of  $\text{O}_2$  in the tissues within the fruit.

In order to determine the effect of coating the surface of the fruit, thereby reducing the permeability of the epidermis, certain lots of all varieties were coated when put in storage. Certain fruit was coated lightly with a non-drying, non-odorous oil, by wiping the surface of the fruit with a well oiled cloth. Other lots were coated with paraffin by wiping them with a solution of paraffin in a volatile solvent, the preparation being one that has been widely advertised for applying to fruit in order to decrease wilting, to improve keeping quality, and to improve general market appearance. Fruits treated both with paraffin and with oil coating, and also check fruits, untreated, have been tested at intervals for hardness, for acidity, for color and general appearance, and for flavor. Respiration experiments in which both oxygen absorbed and carbon dioxide given off were measured, have been carried out at various temperatures.

Results of tests on the rate of softening of apples after coating with paraffin or with oil are somewhat variable, depending upon the variety used. At the end of the storage season coated Delicious apples were about as the checks which received no coating in hardness. Rome Beauty coated fruit was slightly harder than the check apples, York Imperial and Ben Davis were distinctly harder, and Winesap showed even a greater difference in hardness at the end of the season. This difference in hardness of the fruit was apparent when the fruit was withdrawn from 32° F. storage, and became more marked after the fruit had stood for 10 days at a temperature of 60° to 70° F. Apples used as checks were similar in all ways to coated lots, except that they were not coated with any compound. Retardation of softening apparently varied directly with the quantity of oil paraffin on the fruit. If a very heavy coating was applied, the retardation was distinctly greater than if only a light coating was present.

## EFFECT OF COATING UPON THE COLOR CHANGES OF THE FRUIT

Much more marked than the influence of coating the surface on the rate of softening of apples was its influence upon color. Coated fruit showed very little change in the intensity of its green color during the time it was in storage. At the end of the storage season such fruit was very nearly the same shade of green as at the time of placing in storage.

## EFFECT OF COATING UPON THE ACIDITY OF THE FRUIT

Determinations for acidity were made upon coated fruit and upon control fruit throughout the storage season, and there was no consistent variation in acidity due to coating the fruit. Certain varieties showed somewhat higher acidity in coated fruit at the end of the season and others showed less acidity. Because of the wide variation in acidity in individual apples it would be necessary to use large samples to determine accurately whether coating does influence the rate of acidity loss, and fruit was not available in sufficient quantities to determine this more accurately.

## EFFECT OF COATING UPON THE FLAVOR OF THE FRUIT

The flavor of apples that had been coated either with oil or with paraffin varied greatly with the quantity of the coating material present. Unless very heavy coatings had been used, the fruit was of good quality when removed from storage at 32° F. If heavy coating had been used, however, the fruit was stale, flat, and of distinctly unpleasant flavor when removed from 32° F. storage.

After holding for 10 days at a temperature of 60° to 70° F., much of the fruit that was of good quality when removed from a 32° F. room developed undesirable flavors, becoming stale and fermented in taste. Certain varieties, particularly Rome Beauty, became entirely inedible. Some of the very lightly coated fruits maintained a good flavor throughout the period of the tests. These were the fruits that showed a minimum retardation of softening and of color change, and obviously carried very little of the coating material.

The tests so far carried out demonstrate clearly that the ripening processes of apples may be retarded by coating the fruit with substances that reduce the permeability of the epidermis. This retardation is at the expense of flavor, however, unless the coatings be very light. Coatings so light as not to injure the flavor of the fruit will cause only a very slight retardation of the softening process, with a somewhat greater effect upon the color of the fruit.

## RESPIRATION OF COATED AND NORMAL APPLES AT DIFFERENT TEMPERATURES

In order to determine the causes of the retardation of ripening in coated fruit, as well as the cause of the development of bad flavor that may accompany this retardation, the respiration of certain lots of fruit was measured. It is important that both oxygen absorption and CO<sub>2</sub> output be determined, to arrive at a real measure of what is going on in the fruit. In the absence of oxygen, fruit will still give off CO<sub>2</sub> by anerobic respiration, as has been shown by Hill (17) and others. Consequently, the measurement of the oxygen absorption is essential to a knowledge of the source of the CO<sub>2</sub> given off by the fruit.

METHOD OF ESTIMATING CO<sub>2</sub> OUTPUT AND O<sub>2</sub> INTAKE

Most of the work in which both O<sub>2</sub> absorption and CO<sub>2</sub> output have been studied has been carried out by placing fruit in closed chambers, and analyzing the included atmosphere for O<sub>2</sub> and CO<sub>2</sub> at the end of a certain interval. This results in the fruit being exposed to an atmosphere higher in CO<sub>2</sub> pressure and lower in O<sub>2</sub> pressure than the air. The work of Kidd and West (14), as well as investigations reported here, has demonstrated the importance of CO<sub>2</sub> and O<sub>2</sub> concentrations in the ripening of apples.

In order to determine both CO<sub>2</sub> given off and O<sub>2</sub> absorbed without exposing the fruit to varying concentrations of these two gases, the apparatus shown in Figure 14 was used. A large bottle containing a water reserve (A) is connected by a double siphon feed of glass tubing to a second bottle (B). The siphon feed maintains a constant water

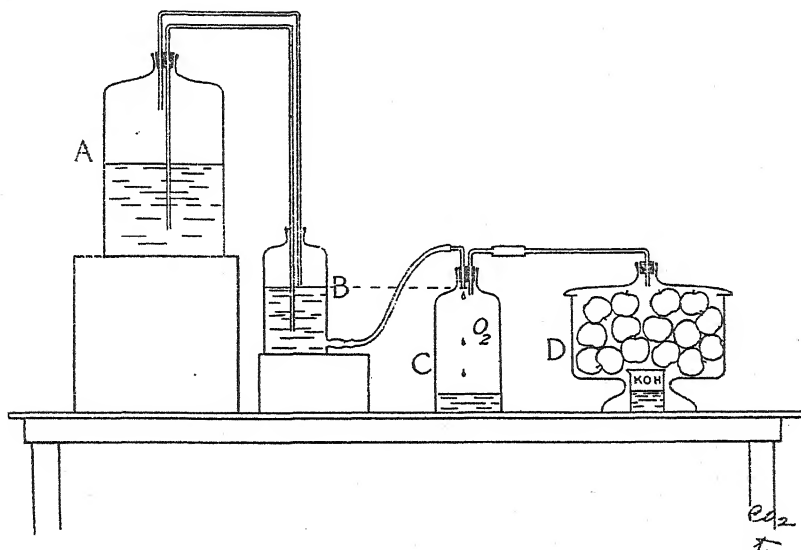


FIG. 14.—Apparatus for determining the amount of CO<sub>2</sub> given off and the amount of O<sub>2</sub> absorbed by the fruit.

level in bottle B. Bottle B has a tubulature at the bottom, by which it is connected, through rubber and glass tubing, to a third bottle, C. Bottle C is full of pure O<sub>2</sub>, being filled by displacement of water. A second tube from bottle C is connected into the closed desiccator D which contains the fruit. In the bottom of the desiccator D is placed a wide beaker containing KOH, a solution approximately twice normal being used.

As the fruit respires O<sub>2</sub> is absorbed by it and CO<sub>2</sub> is given off. The CO<sub>2</sub> given off in the desiccator D is absorbed in the KOH solution in the bottom of the desiccator; a negative pressure consequently develops in the desiccator, due to loss of O<sub>2</sub>. As a result pure O<sub>2</sub> passes over from C, and the loss in O<sub>2</sub> in bottle C is replaced by water which flows from bottle B. It is important that the height of the water level in B be so adjusted that it will be just level with the end of the connection

into bottle C. If this is done, the whole equipment will be under atmospheric pressure at all times.

The oxygen consumption was measured by measuring the volume of water in bottle C and subtracting 3 per cent from this volume as an estimation of the quantity of oxygen absorbed in the water. CO<sub>2</sub> dissolved in the KOH solution was determined by the double titration method as used by Gore (13), phenolphthalein and methyl orange being the indicators used.

Analyses of the contained air in desiccator D showed an accumulation of CO<sub>2</sub> of less than 1 per cent by volume even when held at high temperatures and with rapid respiration going on. The apparatus can be used at any temperature by substituting NaCl or CaCl<sub>2</sub> solutions for the water at temperatures below 32° F.

It is essential that the temperature be kept constant during any run. If the temperature rises, the atmosphere in D and C will expand, driving the water back from C and even with a possible loss of O<sub>2</sub> through B. If the temperature drops, the lowering of the volume of the contained atmosphere will result in a greater volume of water in C and apparent greater O<sub>2</sub> consumption. Fluctuation should not be greater than 1° C.

#### RESPIRATION OF WINESAP APPLES AT 32° F. (0° C.)

The data for the respiration of Winesap apples at 32° F. are presented in Table IV. Oxygen determinations for the first series were lost, but CO<sub>2</sub> output was measured, and both CO<sub>2</sub> and O<sub>2</sub> output were measured for the second series.

TABLE IV.—*Respiration of Winesap apples at 32° F. (0° C.)*

Ex- peri- ment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilo- gram hours.	Weight CO <sub>2</sub> .	CO <sub>2</sub> per kilo- gram hour.	Volume CO <sub>2</sub> per kilo- gram hour.	Volume O <sub>2</sub> per kilo- gram hour.	Respir- atory ratio CO <sub>2</sub> / O <sub>2</sub>
		<i>Grams.</i>		<i>Hours.</i>		<i>Mgm.</i>	<i>Mgm.</i>	<i>Cc.</i>	<i>Cc.</i>	
1	Normal fruit 3 months in storage; check.	2,873	1	358½	1,030.0	2,167.5	2.10	1.07	.....	.....
			2	381½	1,096.0	2,007.1	1.83	.93	1.02	0.91
2	Fruit coated with paraffin.	2,907	1	358	1,040.7	1,807.7	1.74	.89	.....	.....
			2	381½	1,109.0	1,625.9	1.47	.75	.93	.81
3	Fruit coated with oil, light coating.	2,822	1	357½	1,008.9	1,745.4	1.73	.88	.....	.....
			2	381½	1,076.6	1,623.3	1.51	.77	.97	.79
4	Fruit coated with oil, very heavy coating.	2,801	1	357	1,000.0	1,183.6	1.18	.60	.....	.....
			2	381½	1,068.6	1,188.6	1.11	.56	.....	.....

All of the fruit used in experiments 1 to 4 was closely comparable except for the treatments received. It is at once apparent that respiration was markedly reduced by the coatings which the fruit in experiments 2, 3, and 4 received. Light oil coating and paraffin coating both resulted in a marked decrease in CO<sub>2</sub> output as compared with the control, or untreated, fruit. Furthermore, the fact is developed that in the case of these fruits the CO<sub>2</sub> output was not limited by the oxygen supply, for in the coated fruit there was a greater consumption of oxygen per unit of CO<sub>2</sub> output than in the case of the control fruit. This is shown by comparing the respiration ratio, obtained by dividing the volume of CO<sub>2</sub> per unit time and weight of fruit by the volume of O<sub>2</sub> absorbed. The heavily oiled fruit showed an even greater decrease in CO<sub>2</sub> output than did the lightly oiled fruit. Unfortunately, the oxygen absorption record was lost for these heavily coated apples.

At the end of the run samples of the atmosphere from the interior of the apples (from intercellular spaces) were extracted and analyzed. The method employed was that previously described by one of the writers (20). These data, which show the actual atmospheric conditions prevailing inside the fruit, where the respiratory activity takes place, are recorded in Table V.

TABLE V.—Analyses of atmosphere from intercellular spaces of normal and coated Wine-sap apples at 32° F. (0° C.)

Experiment No.	Per cent CO <sub>2</sub> .	Per cent O <sub>2</sub> .	Per cent N <sub>2</sub> by difference.
1. Control; no treatment.....	2.3	18.4	79.3
2. Medium paraffin coating.....	11.1	12.6	76.3
3. Light oil coating.....	10.1	10.4	79.5
4. Heavy oil coating.....	12.4	2.9	84.7

These data show that in all cases there was a marked increase in CO<sub>2</sub> within the tissue following coating and a marked decrease in O<sub>2</sub>. An abundance of oxygen appeared to be present, however, in all lots except those heavily oiled. The fact that the light coatings, which resulted in a large increase of CO<sub>2</sub> in the tissues as compared to the controls, also resulted in a marked decrease in respiratory rate indicates that the CO<sub>2</sub> directly inhibited respiration. The fact that the quantity of O<sub>2</sub> used per cubic centimeter of CO<sub>2</sub> given off was actually greater in the coated fruits than in the controls further indicates that, at least in the lightly coated fruit, the O<sub>2</sub> supply was not the limiting factor in the respiration rate, but rather that respiration was inhibited by CO<sub>2</sub> accumulation. This will be further discussed under the subject of the effect of CO<sub>2</sub> on fruit ripening.

#### RESPIRATION AT 64.5° F. (18° C.)

Magness (20) has shown that the CO<sub>2</sub> in the intercellular spaces of normal apples increases when the fruit is held at higher temperatures, and that the O<sub>2</sub> within the tissues correspondingly decreases. Consequently, the effect of coating the fruit with oil or paraffin would appear to be accentuated at higher temperatures. CO<sub>2</sub> output and O<sub>2</sub> intake were measured on three varieties, and after various treatments, while being held at 64.5° F. (18° C.). Results of these respiration tests are recorded in Table VI.

The data reported in Table VI are of much interest in interpreting the exact effect of coating the surface of the fruit with substances reducing permeability to gases. It will be noted that all cases of coating the fruit (experiments 2, 6, 11, and 12) resulted in greatly decreased CO<sub>2</sub> output, and greatly decreased O<sub>2</sub> absorption as well. Metabolic activity in these lots was reduced to only slightly over half that shown in check lots of fruit. This is in accordance with the slower softening and longer retention of green color in these coated fruits. Also of very marked interest is the respiratory ratio, or ratio of CO<sub>2</sub> given off to O<sub>2</sub> taken up by various lots of fruit. Gerber (12) reported that at 18° C. the respiratory ratio was practically unity in apples. In other words, one molecule of CO<sub>2</sub> was given off per molecule of O<sub>2</sub> absorbed. This would be the case if reduc-

ing sugars were being completely oxidized to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . On the other hand, if  $\text{O}_2$  is not available,  $\text{CO}_2$  will still be formed, as a result of anerobic respiration, or alcoholic fermentation, with a resulting formation of certain products that give a bad flavor to the fruit. The normal oxidation of organic acids to  $\text{CO}_2$  and  $\text{H}_2\text{O}$  will also result in a respiratory ratio superior to 1.

TABLE VI.—*Respiration of apples at 64.5° F. (18° C.)*

Ex- peri- ment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilo- gram hours.	$\text{CO}_2$ given off.	$\text{CO}_2$ per kilo- gram hour.	Vol- ume $\text{CO}_2$ per kilo- gram hour.	Vol- ume $\text{O}_2$ per kilo- gram hour.	Res- pira- tion ratio $\text{CO}_2$ : $\text{O}_2$
		Gm.		Hours.		Mgm.	Mgm.	Cc.	Cc.	
1	Rome Beauty; early pick, (Sept. 14.) held at 32° in unwrapped box until Dec. 18.	2, 338	1	71 $\frac{3}{4}$	167. 6	2, 947. 7	17. 6	8. 96	7. 88	1. 14
			2	93 $\frac{3}{4}$	219. 3	4, 292. 3	19. 6	9. 98	9. 51	1. 05
			3	94 $\frac{3}{4}$	221. 5	4, 077. 0	18. 4	9. 37	8. 28	1. 13
2	Rome Beauty; fruits similar to experiment 1; lightly coated with mineral oil.	2, 240	1	71	159. 0	1, 711. 5	10. 8	5. 50	.....	.....
			2	93 $\frac{3}{4}$	209. 6	1, 904. 2	9. 5	4. 84	.....	.....
			3	95 $\frac{3}{4}$	213. 4	1, 844. 7	8. 6	4. 38	4. 33	1. 01
3	Rome Beauty; similar to experiments 1 and 2 ex- cept held in oiled paper wraps.	2, 218	1	70 $\frac{3}{4}$	156. 4	2, 646. 3	16. 9	8. 60	7. 70	1. 12
			2	93 $\frac{3}{4}$	207. 9	3, 828. 0	18. 4	9. 37	9. 38	1. 00
			3	95 $\frac{3}{4}$	212. 4	3, 728. 3	17. 5	8. 91	8. 85	1. 01
4	Rome Beauty; late pick (Oct. 17); well colored and rather soft.	2, 471	1	69 $\frac{1}{4}$	170. 9	2, 253. 6	13. 2	6. 71	5. 27	1. 28
			2	94 $\frac{3}{4}$	233. 5	3, 443. 3	14. 7	7. 48	7. 41	1. 01
			3	96 $\frac{3}{4}$	238. 5	3, 492. 3	14. 6	7. 43	7. 40	1. 00
5	York Imperial; from oil paper wrapped box held at 32° F. 3 months.	2, 569	1	76	195. 3	2, 553. 5	13. 0	6. 62	.....	.....
			2	94 $\frac{1}{4}$	243. 8	2, 954. 0	12. 1	6. 16	5. 17	1. 19
			3	116 $\frac{1}{2}$	298. 9	3, 359. 8	11. 2	5. 70	5. 64	1. 01
6	York Imperial; similar to experiment 5, but oil coated when put in 32° storage.	2, 528 2, 404	1	96 $\frac{1}{4}$	244. 6	2, 180. 4	8. 9	4. 53	3. 58	1. 21
			2	95 $\frac{1}{4}$	228. 8	1, 841. 6	8. 05	4. 10	3. 08	1. 33
			3	116 $\frac{1}{2}$	280. 1	1, 949. 1	6. 9	3. 51	3. 62	. 99
7	York Imperial; similar to experiments 5 and 6, held in ordinary wrapped box.	2, 580	1	96 $\frac{1}{4}$	249. 0	3, 363. 7	13. 5	6. 87	6. 93	. 99
			2	95 $\frac{1}{4}$	246. 0	2, 893. 2	11. 8	6. 01	5. 51	1. 09
			3	117 $\frac{3}{4}$	301. 2	3, 327. 1	11. 0	5. 60	5. 78	. 97
8	York Imperial; barrel storage.	2, 609	1	96 $\frac{1}{4}$	251. 8	3, 315. 7	13. 2	6. 72	6. 61	1. 01
			2	95 $\frac{1}{4}$	248. 9	2, 695. 8	10. 9	5. 35	5. 34	1. 04
			3	116 $\frac{1}{2}$	304. 6	3, 280. 0	10. 8	5. 30	5. 37	1. 02
9	York Imperial; unwrapped box storage.	2, 667	1	96 $\frac{1}{4}$	256. 5	3, 274. 9	12. 8	6. 32	6. 53	1. 00
			2	95 $\frac{1}{4}$	255. 8	2, 942. 1	11. 1	5. 65	5. 39	1. 05
			3	116 $\frac{1}{2}$	294. 7	3, 352. 8	11. 4	5. 80	5. 85	. 99
10	Winesap; held 3 months at 32° F.; unwrapped box.	2, 709	1	98	265. 5	4, 240. 9	16. 0	8. 14	8. 20	. 99
			2	116 $\frac{1}{2}$	315. 3	5, 372. 8	17. 0	8. 65	8. 04	1. 07
			3	97 $\frac{1}{2}$	254. 1	2, 756. 3	10. 8	5. 50	5. 16	1. 07
11	Winesap; similar to exper- iment 10, but oil-coated when removed to 64.5° F.	2, 606	1	117	305. 1	3, 308. 4	10. 8	5. 50	4. 51	1. 22
			2	.....	.....	.....	.....	.....	.....	.....
			3	.....	.....	.....	.....	.....	.....	.....
12	Winesap; fruit paraffin- coated when put in at 32°.	2, 298	1	98 $\frac{1}{4}$	226. 4	2, 172. 6	9. 6	4. 89	4. 48	1. 09
			2	110 $\frac{1}{2}$	266. 1	2, 776. 4	10. 4	5. 29	4. 27	1. 24
			3	.....	.....	.....	.....	.....	.....	.....
13	Winesap; held in wrapped box; no treatment.	2, 723	1	96 $\frac{1}{4}$	262. 1	4, 184. 7	16. 0	8. 14	8. 07	1. 01
			2	118 $\frac{3}{4}$	323. 4	5, 439. 0	16. 8	8. 55	7. 82	1. 09
			3	.....	.....	.....	.....	.....	.....	.....

TABLE VII.—*Composition of intercellular atmosphere of Rome Beauty apples held at 64.5° F. (18° C.)*

Res- pira- tion experi- ments No.	Description of fruit.	$\text{CO}_2$ .	$\text{CO}_2$ .	Per cent $\text{N}_2$ by difference.
		Per cent.	Per cent.	
1	Early pick, untreated. ....	4. 95	14. 7	80. 35
2	Early pick, oil coated. ....	15. 8	1. 9	82. 3
4	Late pick, colored ripe. ....	6. 8	15. 5	77. 7



At the temperature here studied (64.5° F. or 18° C.) the respiratory ratio in all untreated fruit was fairly close to unity, averaging slightly above. Coated fruit averaged a somewhat higher respiratory ratio. Experiments 6, 11, and 12 show an average ratio of 1.16, while checks for York Imperial and Winesap runs averaged 1.04. Data on the O<sub>2</sub> absorption of oil-coated Rome Beauty apples were lost. Consequently, at 64.5° F. it appears that the oxygen supply was somewhat depleted in coated fruits as compared to untreated fruit, and that there was the beginning of anerobic respiration with resulting bad flavor.

Only data for the analyses of the intercellular atmosphere of Rome Beauty apples held under these tests are available, but they bear out the suggestion that the oxygen supply was very largely depleted in the coated fruit. These data are reported in Table VII.

From these results, which represent averages of a number of determinations, it is apparent that coating the surface of the fruit had so reduced available oxygen as to render anerobic respiration probable, whereas untreated fruit had an abundance of oxygen within the tissues. Data in Table VI showing a greater CO<sub>2</sub> output per unit O<sub>2</sub> intake in oil-coated than in untreated fruit indicate that anerobic respiration was already occurring to a limited extent.

This conclusion is further borne out by results reported in Table VIII, which records respiration data for Winesap and Rome Beauty apples held at a temperature of 80° F. (26.5° C.).

TABLE VIII.—*Respiration of apples at 80° F. (26.5° C.)*

Ex- peri- ment No.	Description of fruit.	Weight of fruit.	Run No.	Length of run.	Kilo- gram hours.	Weight of CO <sub>2</sub>	CO <sub>2</sub> per kilo- gram hour.	CO <sub>2</sub> per kilo- gram hour.	O <sub>2</sub> per kilo- gram hour.	Respi- ratory ratio CO <sub>2</sub> O <sub>2</sub> .
		<i>Gms.</i>		<i>Hours.</i>		<i>Mgm.</i>	<i>Mgm.</i>	<i>Cc.</i>	<i>Cc.</i>	
1	Winesap; after 4 months at 32° F.; unwrapped box.	2,740	1	70	191.8	4,786.6	24.95	12.70	11.65	1.09
			2	69½	191.1	4,404.7	23.05	11.73	12.24	.96
2	Winesap; similar to experiment 1, except coated with mineral oil when removed to 26.5° C.	2,650	1	70	185.5	3,207.5	17.3	8.81	4.20	2.15
			2	69½	184.8	2,812.8	15.2	7.74	5.76	1.34
3	Winesap; same as experiment 1.	2,637	1	69½	184.1	4,470.8	24.3	12.37	11.43	1.08
			2	69	184.4	4,173.8	22.6	11.50	12.12	.95
4	Rome Beauty; early pick; after 4 months at 32° F.; no treatment.	3,113	1	69½	216.9	5,755.3	26.5	13.49	12.89	1.05
		3,005	2	69½	210.0	4,978.6	23.7	12.06	12.88	.94
5	Rome Beauty; similar to experiment 3, except oil-coated when removed to 26° C.	2,525	1	69½	176.1	3,563.9	20.2	11.20	4.57	2.45
			2	69½	176.3	2,937.6	16.7	8.50	7.15	1.19

Experiments 1 and 3 show normal Winesap apples under test at 80° F., with experiment 2 showing similar fruit oil coated when the test was started. Normal fruit has a respiratory ratio averaging 1.025 and 1.015 for experiments 1 and 3, respectively, while the oil-coated fruit gave a ratio of 1.75. Results on Rome Beauty were very similar. The rate of CO<sub>2</sub> evolution in both varieties was reduced from normal only about 35 per cent by the oil coating. This reduction was no greater in proportion than that at 64.5° (see Table VI). At the higher temperature, however, oxygen could not enter the oil-coated fruit sufficiently rapidly to supply the need, and anerobic respiration resulted. Analyses for the internal atmosphere of some of the fruits held at 80° F. are recorded in Table IX.

TABLE IX.—Composition of intercellular atmosphere of apples held at 80° F. (26.5° C.)

Respiration experiment No. (Table VIII).	Description of fruit.	CO <sub>2</sub> .	O <sub>2</sub> .	Per cent N <sub>2</sub> by difference.
		<i>Per cent.</i>	<i>Per cent.</i>	
1, 3	Winesap, untreated.....	9.2	14.2	76.6
2	Winesap, oil coated.....	14.0	1.7	84.3
4	Rome Beauty, untreated.....	5.5	16.2	78.3
5	Rome Beauty, oil coated.....	18.8	2.9	78.3

Analyses for oxygen in the fruit held at 80° F., where the respiratory ratio was much superior to unity, showed about the same quantity present as in similar fruit held at 64.5° F., where the anerobic respiration apparently was just starting, and apparently represents about the concentration present when oxygen is the limiting factor in fruit respiration.

The total quantity of oxygen entering the oil-coated fruit at 64.5° F. was approximately the same as the total amount entering at 80° F. It appears, therefore, that in fruit coated as heavily as the fruit here tested about 60° marked the highest temperature at which the oxygen supply entering the fruit was sufficient to prevent anerobic respiration. When held at temperatures above 60° anerobic respiration was very pronounced.

#### DISCUSSION OF EFFECT OF COATING SURFACE OF FRUIT UPON THE RIPENING PROCESSES

From the data presented above it is apparent that coating the surface of the fruit with such substances as oil or paraffin tends to retard respiration, regardless of the temperature at which the fruit is held, and thus to retard the ripening processes. The degree of this retardation will vary with the thickness of the coating. Apples heavily coated with either oil or paraffin are retarded in ripening much more than apples lightly coated.

So long as the substances used in coating the fruit are entirely tasteless and odorless, the flavor appears to be normal until anerobic respiration sets in. Anerobic respiration may occur in fruit held at 32° F., if the coating is very heavy. The higher the temperature at which the fruit is held the less the coating that will be required to induce anerobic respiration. At 64.5° fruit lightly coated appeared to show just the beginning of anerobic respiration, while the respiration of similarly treated fruit when held at 80° F. was largely anerobic. In fact it seems highly probable that anerobic respiration will occur in certain fruits at very high temperatures without any coating of the surface. Taylor and Overholser (26) reported that very high temperatures inhibited the ripening of Bartlett pears, with associated loss of quality. It seems probable that anerobic respiration occurred. Gerber's high respiratory ratio on apples held at very high temperatures (30° and 33° C.) may have been due to oxygen depletion in the tissues.

There is a wide variation in the amount of oxygen in the tissues of different varieties of apples under similar conditions. Magness and

Burroughs<sup>9</sup> found 5 to 6 per cent oxygen in western Winesap apples at 68° and 11 to 14 per cent in New York Baldwin apples under the same conditions. Table IX shows 14 per cent oxygen in Winesap apples grown in Virginia even at a temperature of 80°. Magness (20) found only 5.5 per cent oxygen in the tissues of California-grown Yellow Newtown apples at 68° F. (20° C.), while Virginia-grown Ben Davis showed 13 to 14 per cent oxygen at 80° F., and Delicious 16 to 17 per cent. Thus it is apparent that the internal atmosphere will vary with the skin permeability, which in turn will vary with the variety and the conditions under which it is grown.

The fact appears definitely established that coating the surface of the fruit, either with paraffin or with oil, will somewhat retard the ripening of the fruit. If too much is applied, however, anerobic respiration will result, and the flavor of the fruit will be injured. The amount that can be applied without injury will vary with the variety and with the temperature to which the fruit will be exposed. Very thin coatings may result in bad flavor in certain varieties, particularly when these varieties are exposed to the relatively high temperatures usually encountered by apples before ultimate consumption.

The effect of coating appears to be twofold. At certain temperatures there seems to be an actual limitation in the oxygen supply which not only retards ripening but also results in bad flavor. At all temperatures there is an apparent increase in the concentration of CO<sub>2</sub> within the tissues, and this increased concentration of CO<sub>2</sub> seems directly to inhibit the ripening processes, as will be discussed later.

#### INFLUENCE OF CARBON DIOXIDE CONCENTRATIONS UPON THE RIPENING OF APPLES

It has long been known that increasing the carbon dioxide pressure in the atmosphere surrounding plant tissues would have a marked effect upon their metabolism. Brooks, Cooley, and Fisher (3) reported that apples held in concentrations of 5 per cent and upward of CO<sub>2</sub> remained firm and green, but developed an alcoholic flavor. More recently, Kidd and West (14) have carried on extensive tests on storing apples in CO<sub>2</sub> with diminished O<sub>2</sub> pressures. Fruit has been stored in tight chambers and the chambers kept closed until the O<sub>2</sub> in the air was reduced to from 5 to 8 per cent, and the CO<sub>2</sub> reached 15 per cent, due to the respiration of the fruit. Then sufficient ventilation was given to maintain this ratio. They found it important not to reduce the O<sub>2</sub> pressure below 5 per cent or to increase the CO<sub>2</sub> above 15 per cent. They report that apples so handled keep about twice as long as controls held in air.

In order to determine the influence of CO<sub>2</sub> pressure, as distinct from limitation of O<sub>2</sub>, upon the rate of ripening of apples, preliminary experiments were made with holding apples in various concentrations of CO<sub>2</sub>. The apparatus used is shown in Figure 15. It was desirable not to keep the fruit in a closed chamber, where respiration from the fruit itself would alter the composition of the surrounding air. Consequently, two large bottles were used, A and B, each of 19 liters capacity. Bottle A was filled with water. In B the desired gas mixture was secured by introducing pure CO<sub>2</sub>, O<sub>2</sub>, and air in the desired amounts, using water displacement to measure the volumes. A layer of mineral oil was left in the bottom of this bottle to reduce unequal absorption of

<sup>9</sup> MAGNESS, J. R., and BURROUGHS, A. M. OP. CIT.

the gases by water. The bottle was closed with a stopper containing a tube (*t*) extending to the bottom of the bottle, and with another tube extending from the top of the bottle B to the desiccator C containing the apples under test. A siphon from A entered the top of the tube *t*, which delivered under the oil in B. As water flowed through this siphon into the bottom of bottle B, the contained gases passed out and through the desiccator C. They were delivered into the bottom of C, and escaped through a capillary opening in the top of C.

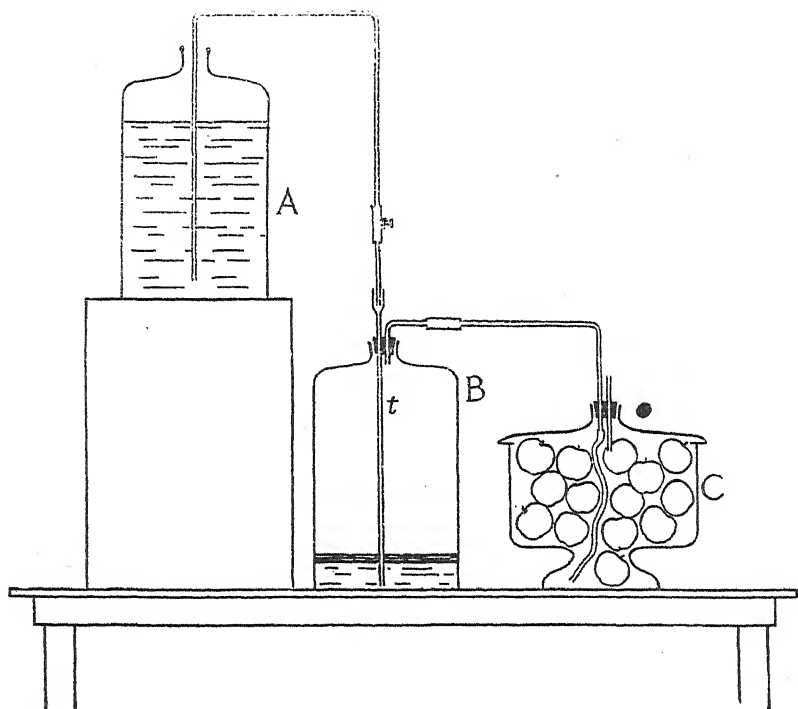


FIG. 15.—Apparatus for determining the influence of  $\text{CO}_2$  pressure upon the rate of ripening of apples.

In order to determine whether or not any variation found was due to  $\text{CO}_2$  present rather than to a deficiency of  $\text{O}_2$ , the  $\text{O}_2$  content of the atmosphere in all tests was kept at approximately 20 per cent, or normal air concentration.  $\text{CO}_2$  concentrations were varied from 0 to 50 per cent.

Some variations occurred in the rate of flow of the gas through the desiccators containing the apples. In all cases, however, about 18 liters of gas passed through per 24 hours, so there were never more than slight accumulations of  $\text{CO}_2$  or deficiencies in  $\text{O}_2$  due to the respiration of the fruit.

Two sets of experiments were run, one on Winesap and one on Delicious apples. Pressure tests, to determine the relative rates of softening, were made on a representative sample of the fruit at the beginning of the test and on all the various lots at the end of the test. Acidity determinations were also made on all lots. Data for Winesap apples are recorded in Table X.

TABLE X.—*Relative hardness and acidity in Winesap apples after being held 10 days at 71.5° F. (22° C.) in various concentrations of CO<sub>2</sub>.*<sup>a</sup>

Description of test.			Pressure test.	Acidity— cc. N/10 acid per 10 gm. wet weight.
Lot No.	Per cent CO <sub>2</sub> .	Per cent O <sub>2</sub> .		
1.....	0	20	12.37	6.55
2.....	5	20	13.07	6.41
3.....	10	20	13.52	6.22
4.....	20	20	13.48	6.36
5.....	50	20	14.22	6.27
6.....	100	0	14.78	6.14

<sup>a</sup> Removed from 32° F. at beginning of test. Pressure test, 15.37 pounds. Acidity, 6.53 cc. N/10 per 10 gm. of wet tissue when removed from 32° F.

Internal atmospheres were analyzed from 5 apples of each lot at the end of the test. Oxygen present in all fruits (except lot No. 6, not analyzed) ranged from 6 to 13 per cent, while CO<sub>2</sub> ranged approximately 20 per cent above the concentration of the surrounding atmosphere. These analyses indicate that there was in all lots an abundance of O<sub>2</sub> within the tissue.

There was a marked relationship between CO<sub>2</sub> concentration and the rate of softening of the fruit. An atmosphere containing even 5 per cent CO<sub>2</sub> resulted in a distinctly slower rate of softening than did air of the normal concentration, while higher concentrations resulted in still further decreasing the softening rate. Acidity, on the other hand, apparently, disappeared more rapidly at the higher concentrations of CO<sub>2</sub>. While the acidity results are all so close together that they are not at all conclusive, the uniformity of the fruit used in these tests as well as the regularity of the results makes them very suggestive. Results of a similar series of tests on Delicious apples are recorded in Table XI.

TABLE XI.—*Relative hardness and acidity in Delicious apples after being held 11 days at 71.5° F. (22° C.) in various concentrations of CO<sub>2</sub>.*<sup>a</sup>

Description of test.			Pressure test.	Acidity— cc. N/10 acid per 10 gm. wet tissue.
Lot No.	Per cent CO <sub>2</sub> .	Per cent O <sub>2</sub> .		
			<i>Pounds.</i>	
1.....	0	20	11.78	2.26
2.....	5	20	12.31	2.43
3.....	10	20	12.63	2.31
4.....	20	20	13.82	2.21
5.....	50	20	13.58	2.26
6.....	100	0	13.58	2.22

<sup>a</sup> Removed from storage at 32° F. at beginning of test. Pressure test, 13.68 pounds. Acidity, 2.36 cc. N/10 acid per 10 grams of wet tissue when removed from storage at 32° F.

The decreased rate of softening of Delicious when held in various concentrations of  $\text{CO}_2$  was even more marked than that for Winesap. There was little softening in any lot held in concentrations of  $\text{CO}_2$  of 20 per cent or more. Results for acidity change are less pronounced. The results as a whole for Delicious are rather less dependable than those for Winesap because of the lack of uniformity in the samples used.

#### EFFECT OF $\text{CO}_2$ CONCENTRATION ON FLAVOR

A number of apples from each of the above tests were tested for flavor. Concentrations of 5 and 10 per cent  $\text{CO}_2$  gave no flavor that was distinctive from that of control lots held in air. In the lot held in 20 per cent  $\text{CO}_2$ , most apples were normal, though in some fruit there was a suggestion of a flavor resulting from anerobic respiration. Apparently, 20 per cent  $\text{CO}_2$  marked the extreme concentration in which apples can be held without serious impairment of flavor. Fruit held in 50 per cent  $\text{CO}_2$  with 20 per cent  $\text{O}_2$  as well as fruit held in 100 per cent  $\text{CO}_2$  was entirely inedible. It is of much interest to note that the flavor produced in the apples in high  $\text{CO}_2$  concentration, even in the presence of abundant  $\text{O}_2$  in the tissues, is very similar to that produced by partially sealing the fruit with oil or paraffin, and in many fruits was very similar to the flavor of water-cored apples.

#### GENERAL DISCUSSION OF THE EFFECT OF COATING FRUIT, AND $\text{CO}_2$ CONCENTRATIONS, UPON THE RIPENING PROCESSES

It seems well established from the preceding data that coating the fruit with any material which retards gaseous exchanges will tend to retard softening and the development of a yellow color. This treatment results in a higher concentration of  $\text{CO}_2$  within the tissues as well as a decreased  $\text{O}_2$  supply. At lower temperatures, coating the fruit moderately does not result in a lack of oxygen within the tissues, but the higher concentration of  $\text{CO}_2$  appears to directly retard the ripening processes. At higher temperatures both  $\text{CO}_2$  accumulation and  $\text{O}_2$  deficiency may take part in retarding the ripening process.

The fact that high concentrations of  $\text{CO}_2$ , even in the presence of oxygen, gives a flavor to the fruit similar to that resulting from an absence of  $\text{O}_2$ , suggests that the action of the  $\text{CO}_2$  may be to inhibit the oxydizing enzymes, and thus to stop or retard normal oxidation.

#### INFLUENCE OF OILED PAPER WRAPS UPON RIPENING PROCESSES

There is a widespread belief in the commercial apple trade that wrapping the apples in oiled papers, as has been extensively done for the control of storage scald, results in a slower ripening of the fruit and a prolonging of the storage life (7). The above data on coating the fruit with oil or paraffin are very suggestive in this connection.

The papers that have been used for wrapping to control scald carry a very high oil content. The oil will leave the paper very readily, as may be shown by placing an oiled paper over one that contains no oil. The oil will quickly spread to the non-oiled paper. The waxy coating, or cuticle, of apples appears to be exceedingly soluble in mineral oil. Consequently, heavily oiled paper loses a portion of its oil to the fruit wax. It has been noted that where sufficient oil has been used to control scald there is often a distinct appearance of oiliness on the fruit surface.

While the oil coating thus secured would probably never be sufficient to result in bad flavor in the fruit, due to its inducing anerobic respiration, it undoubtedly differs in degree only from the effect produced by the heavier oil coating secured by wiping the fruit with a well oiled cloth, the method followed in the "oiled coating" here discussed. In the storage tests of holding fruit in oiled papers in connection with this work, small-sized wrappers were used, and there was apparently little, if any, oil transferred to the fruit. Under these circumstances no variation in rate of ripening in storage could be detected between fruit not wrapped and fruit wrapped in oiled paper. The wraps used, however, also failed to control the development of storage scald, although containing a very appreciable quantity of oil. The results obtained with this paper, together with the general appearance of fruit on which storage scald has been controlled by oiled wraps, strongly suggests that some oil must be transferred from the wrapper to the fruit if scald control is to be effective. In that case, the ripening of the fruit would be somewhat retarded by the use of oiled wraps.

#### SUMMARY

Changes in apples as they approach the condition of ripeness on the tree have been studied. These changes include (1) increase in size, (2) increase in the area and intensity of the red color and the change in the green or ground color from leaf green to yellow green, (3) a progressive softening of the fruit, and (4) a decrease in the apparent acidity in the fruit. Fruit softened very rapidly while still on the trees at Arlington, Va., during the warm, dry autumn of 1922.

Changes in the fruit following picking are primarily a continuation of pre-picking changes. Softening, acidity change, sugar change, etc., continue after picking much as while the fruit is still on the tree. The rate of these changes varies with the temperature at which the fruit is held.

Rate of softening of apples while in 32° F. storage as compared with storage at 70° F. varies with varieties. Ben Davis softened as much in 2½ months at 32° F. as in 12 days at 70° F., Winesap and Rome Beauty as much in 3 to 4 months at 32° F. as in 12 days at 70° F., York Imperial as much in about 5 months at 32° F. as in 12 days at 70° F., while Delicious and Rhode Island Greening were softer at the end of 12 days at 70° F. than they were at the end of their storage period, about 6 months, when placed in 32° storage immediately after picking.

All varieties used showed a constant decrease in acidity during the time the apples were held in 32° storage. Rate of decrease was very nearly the same in all varieties, regardless of acid content. Thus Delicious, with an initial acid content of about 3 cubic centimeters N/10 acid per 10 grams of wet tissue, lost acid until after 6 months only 2 cubic centimeter N/10 acid was present, a loss of from 30 to 40 per cent. Rhode Island Greening, with an acid content equivalent to 9 cubic centimeters N/10 acid at the beginning of the season, also lost only about 1 cubic centimeter N/10 acid, or about 12 per cent. Range of varieties tested, in order of decreasing acid content, is Rhode Island Greening, Ben Davis, York Imperial, Winesap, Rome Beauty, and Delicious. Percentage loss of acid during 6 months in storage is in the reverse order, those varieties showing lowest total acidity showing highest percentage loss.

Coating the surface of the fruit, either with paraffin or with oil, reduces the permeability of the fruit skin in proportion to the amount of the coating material applied. This results in higher  $\text{CO}_2$  concentration, and lower  $\text{O}_2$  concentration, in the gas present in the intercellular spaces of the fruit. If too much of the coating material is applied, anaerobic respiration will result, with the development of disagreeable flavors in the fruit.

Increasing the concentration of  $\text{CO}_2$  in the atmosphere surrounding apples results in a slower rate of softening of the apples. Coating the surface of the fruit with paraffin or oil also results in a slower rate of softening both in storage at  $32^\circ\text{F.}$  and at  $70^\circ\text{F.}$ , probably by increasing the  $\text{CO}_2$  concentration within the tissues.

Coating the fruit, either with paraffin or oil, resulted in a reduced respiration rate, whether the fruit was being tested at  $32^\circ\text{F.}$  ( $0^\circ\text{C.}$ ), at  $64.5^\circ\text{F.}$  ( $18^\circ\text{C.}$ ), or at  $80^\circ\text{F.}$  ( $26.5^\circ\text{C.}$ ); at  $32^\circ\text{F.}$  the ratio  $\frac{\text{CO}_2}{\text{O}_2}$  was less than 1, indicating that there was an abundance of  $\text{O}_2$  within the tissue, and that the reduced respiration rate was due to  $\text{CO}_2$  accumulation. At  $64.5^\circ\text{F.}$ ,  $\text{O}_2$  within the tissues was practically exhausted, and some anaerobic respiration apparently occurred in coated fruit, for the respiratory ratio  $\frac{\text{CO}_2}{\text{O}_2}$  was greater than 1. At  $80^\circ\text{F.}$  the ratio  $\frac{\text{CO}_2}{\text{O}_2}$  was much greater than 1, indicating marked anaerobic respiration.

In normal, uncoated fruit the respiratory ratio at all temperatures was approximately 1, indicating that there was no anaerobic respiration in any of the normal fruit studied. Rate of  $\text{CO}_2$  evolution in normal Winesap apples averaged 1.97 milligrams per kilogram hour at  $0^\circ\text{C.}$ , 16.45 milligrams at  $18^\circ\text{C.}$ , and 23.73 milligrams at  $26.5^\circ\text{C.}$  This appears to be very close to the ratio of the softening rates at the different temperatures.

Atmospheres of  $\text{CO}_2$  in concentrations of 5 per cent, 10 per cent, 20 per cent, and 50 per cent, with 20 per cent  $\text{O}_2$ , markedly inhibited the softening rates of apples, the retardation in softening rate varying with the  $\text{CO}_2$  concentration. Five per cent and 10 per cent concentrations of  $\text{CO}_2$  had no appreciable effect upon the flavor of apples. In concentrations of 20 per cent  $\text{CO}_2$  there was a very slight flavor of fermentation. Fruit held in 50 per cent  $\text{CO}_2$  was entirely inedible. These tests were all made at  $71.5^\circ\text{F.}$  ( $22^\circ\text{C.}$ ). They indicate that any concentration of  $\text{CO}_2$  in which men can work, such as the concentrations of 2 to 3 per cent which may occasionally occur in cold-storage rooms, will not injure the fruit, and may, in fact, be distinctly beneficial, through retarding the softening process.

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# A STUDY OF THE EFFECTS OF PUMPKIN SEEDS ON THE GROWTH OF RATS<sup>1</sup>

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## INTRODUCTION

The object of the experiment recorded in this paper was to investigate the belief prevalent among stock feeders as to the injury caused by of *Cucurbita pepo* (pumpkin) seeds in animal metabolism and to verify the results obtained by scientific investigators in the study of the effects of these seeds upon animals.

Pott<sup>3</sup> states that the claim made that pumpkin seeds are injurious is without foundation.

Lindsey<sup>4</sup> found that the seeds appeared to have no injurious effects upon animals when fed in the amounts found in the entire pumpkin fruit.

The writer<sup>5</sup> fed pumpkins with increased quantities of *Cucurbita* seeds to growing pigs and found no detrimental effect upon the gain in body weight of the animals. Elsewhere he<sup>6</sup> experimented on himself by including *Cucurbita pepo* seeds in his diet. In studying the kidney excretion he found that the rôle played by pumpkin seeds in animal metabolism is of a chemico-pharmacognostic value.

## EXPERIMENT

Six rats of the same litter resulting from the crossing of a pure Norwegian buck and an albino doe of the "Tyler" strain were used in this experiment. The foundation stocks of both the Norwegian and albino strains were purchased from the Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

The experimental animals were born April 24, 1923, and weaned May 18. They were all males and at the beginning of the feeding experiment, June 22, were 60 days old and of the following weights:

No. of group.	No. of animal.	Sex.	Weight.
			Gm.
I.....	41	♂	135
	43	♂	137
II.....	45	♂	125
	46	♂	127
III.....	40	♂	118
	42	♂	134

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The balanced basal ration consisted of the following ingredients:

	Gm.		Gm.
Wheat.....	2,900	Meat scraps.....	1,000
Corn.....	2,800	Cod liver oil.....	300
Oats (rolled).....	750	Calcium carbonate.....	150
Green peas.....	750	Sodium chlorid.....	100
Alfalfa (flour).....	500		

The pumpkin seeds used in this experiment were obtained from a seed store. All the grain was milled very fine and thoroughly mixed.

Up to June 22 all experimental animals were kept on the same basal balanced ration, and beginning with June 1 their gain in weight was recorded weekly. On June 22 the animals were arranged for the feeding trial in the following way: Three groups of rats, two animals in each group, were placed in three separate cages and fed on different grain mixtures. Group I, No. 41 and 43, were fed on the basal balanced ration and served as a control in this experiment; Group II, No. 45 and 46, received a grain mixture consisting of 50 parts ground pumpkin seeds and 50 parts of the basal balanced ration; Group III, No. 41 and 42, were fed ground pumpkin seeds only. The food was given without restriction and a constant supply of fresh tap water was kept before the animals.

The all-wire cages were provided with false bottoms wherein a thin layer of wooden shaving served as bedding for the animals. The litter was removed weekly, the false bottoms disinfected, and clean bedding was provided for the animals. Weekly records of body weight of the rats were kept until the completion of the feeding trial. The results are given in the accompanying tables:

TABLE I.—Pumpkin seed experiment

No. of group.	No. of animal.	Initial weight.	June 8 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	June 16 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	June 22 weight.	Total gain per 100 gm.	Daily gain per 100 gm.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I a.....	41	68	88	.....	.....	117	.....	.....	135	.....	.....
	43	67	87	+29.5	+4.22	118	+34.2	+4.27	137	+15.7	+2.61
II b.....	45	65	85	.....	.....	110	.....	.....	125	.....	.....
	46	73	89	+26.0	+3.71	113	+28.1	+3.51	127	+13.0	+2.16
III c.....	40	66	82	.....	.....	106	.....	.....	118	.....	.....
	42	68	88	+26.8	+3.82	118	+31.7	+3.96	134	+12.5	+2.08

a Control; fed balanced ration.

b Fed 50 parts pumpkin seed and 50 parts balanced ration.

c Fed pumpkin seeds only.

TABLE I.—Pumpkin seed experiment—Continued

No. of group.	No. of animal.	Initial weight.	June 30 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	July 16 weight.	Total gain per 100 gm. live weight.	Daily gain per 100 gm. live weight.	July 14 weight.	Total gain per 100 gm.	Daily gain per 100 gm. live weight.	July 20 weight.	Total gain per 100 gm. live weight.	Daily gain per 100 gm. live weight.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I a.....	41	135	168	.....	.....	195	.....	.....	215	.....	.....	234	.....	.....
	43	137	170	+24.2	+3.02	200	+16.8	+2.8	235	+13.9	+1.73	263	+10.4	+1.73
II b.....	45	125	172	.....	.....	192	.....	.....	220	.....	.....	245	.....	.....
	46	127	173	+36.9	+4.60	195	+12.17	+2.03	223	+14.4	+1.8	243	+9.93	+1.65
III c.....	40	118	149	.....	.....	171	.....	.....	190	.....	.....	197	.....	.....
	42	134	159	+22.2	+2.77	182	+14.6	+2.43	200	+10.4	+1.3	213	+5.12	+0.85

No. of group.	No. of animal.	Initial weight.	July 27 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	Aug. 3 weight.	Total gain per 100 gm.	Daily gain per 100 gm.	Aug. 10 weight.	Total gain per 100 gm.	Daily gain per 100 gm.
		Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.	Gm.
I a.....	41	234	251	.....	.....	270	.....	.....	288	.....	.....
	43	263	285	+7.84	+1.12	310	+8.20	+1.17	327	+6.03	+0.89
II b.....	45	245	275	.....	.....	300	.....	.....	317	.....	.....
	46	242	260	+9.85	+1.40	278	+8.03	+1.14	297	+6.22	+0.88
III c.....	40	197	183	.....	.....	200	.....	.....	208	.....	.....
	42	213	220	-1.70	-0.24	216	+3.22	+0.46	220	+2.88	+0.41

a Control; fed balanced ration.

b Fed 50 parts pumpkin seeds and 50 parts balanced ration.

c Fed pumpkin seeds only.

## DISCUSSION

From the tables here presented it will be found that calculations were made by subtracting the weekly weight of each animal from the

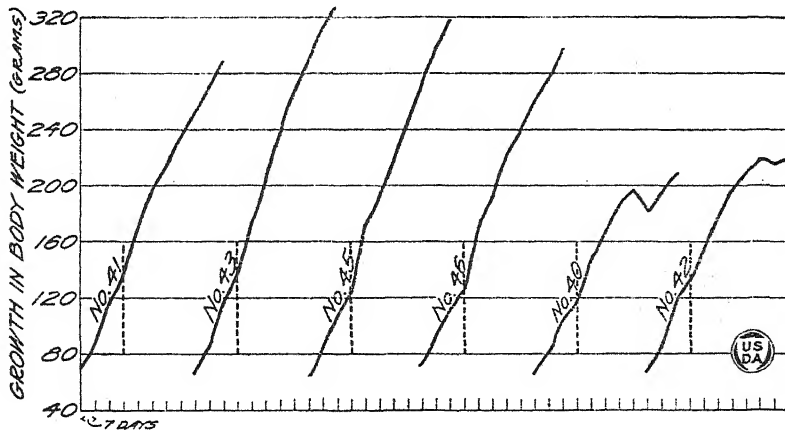


FIG. 1.—No. 41 and 43 belong to Group I, which were fed the basal balanced ration. No. 45 and 46 belong to Group II and received 50 parts of ground pumpkin seeds for every 50 parts of the basal balanced ration. No. 40 and 42 constitute Group III, which were fed ground pumpkin seeds only. The curves represent the growth in body weight of the rats beginning June 1 and ending August 10. The broken lines pass through the points at which the feeding trial of pumpkin seeds started.

preceding weekly weight of the same animal. The total gain constituted the algebraic sum of the differences of all the animals in each group. The total gain per 100 gm. body weight was obtained by divid-

ing the total weekly gain by the total body weight of each group of the preceding week and multiplying the quotient by 100. The daily gain per 100 gm. body weight was derived by dividing the total weekly gain per 100 gm. body weight by the number of days in the week.

In studying the figures and the records given above it will be noticed that the change of environment and ration shortly after grouping of the experimental animals was most pronounced in Group II, i. e., on June 30

the daily gain per 100 gm. body weight for Group II was 1.83 gm. greater than that of Group III and 1.58 gm. greater than that of the control, Group I.

As will be noted from Figure 1, the animals of Group II show smooth growth curves similar to those of the control, while the animals in Group III

depart considerably, a break being noticeable in their growth curves when compared either with those of Group I or Group II.

Figure 2 shows the stimulating effect of pumpkin seeds when mixed with the basal ration, 50 per cent of each, as fed to Group II. It also demonstrates the poor effect of pumpkin seeds, as the sole food, on gain in weight of growing rats.

#### SUMMARY

A seven-weeks feeding-trial of pumpkin seeds (*Cucurbita pepo*) was conducted with six rats of the same age and of the same litter. The animals were arranged in three separate groups; Group I received a grain mixture constituting the basal balanced ration; Group II received 50 parts of ground pumpkin seeds to every 50 parts of the basal balanced grain mixture; and Group III was fed on ground pumpkin seeds only. The results obtained in the experiment suggest the following conclusions:

Pumpkin seeds fed in as high a quantity as 50 per cent of the grain mixture show no injurious effects upon the growth of rats.

Rats fed only on pumpkin seeds exhibit poor growth.

A pronounced increase was noted in the daily gain of body weight in Group II during the first week, this being 1.58 gm. greater than that of Group I, the control, and 1.83 gm. greater than the gain of Group III.

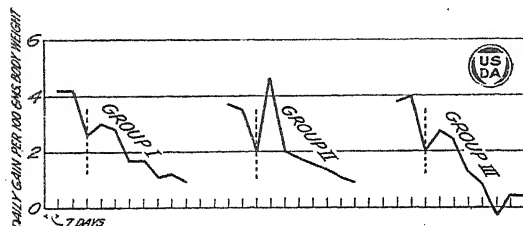


FIG. 2.—The curves represent the daily gain per 100 gm. body weight of each group of rats beginning June 1 and ending August 20. The broken lines pass through the points at which the feeding trial of pumpkin seeds started.

# THE ARGUS TORTOISE BEETLE<sup>1</sup>

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## INTRODUCTORY

The foliage of sweet potato, wherever planted in the United States, is attacked every year by tortoise beetles of different species, the largest of which is known as the Argus tortoise beetle (*Chelymorpha cassidea* Fab.).<sup>2</sup> This species breeds on convolvulaceous plants, and, until the year 1919, was rather generally believed to be more commonly found on bindweed (*Convolvulus* spp.) or wild morning-glory (*Ipomoea* spp.)

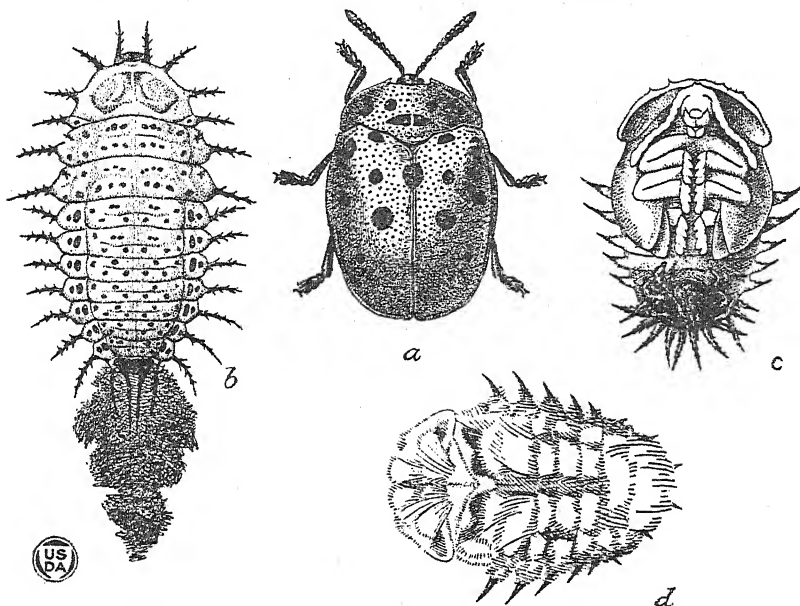


FIG. 1.—The Argus tortoise beetle: a, Beetle; b, larva with feci-fork extended at end; c, pupa, ventral view; d, dorsal view of pupa with characteristic covering. Greatly enlarged.

and related wild plants than on sweet potato. That year the species was abundant and attracted more attention on sweet potato (*Ipomoea batatas*) than on wild plants, and recent studies tend to show that it prefers the cultivated plant, even when wild Convolvulaceae are available in the immediate vicinity. Thus far, however, it has not been a pest of importance.

## DESCRIPTION

### THE BEETLE

The beetle (fig. 1, a, and Pl. 1, A, a) is dark brick red when fully mature, and, before it is fully colored, of different shades of yellow. Its

<sup>1</sup> Accepted for publication, Nov. 1, 1923.

<sup>2</sup> Formerly known as *Chelymorpha argus* Licht.; order Coleoptera, family Chrysomelidae.

upper surface is ornamented with black dots of variable number and size, the variation being largely dependent on locality. Usually, in the eastern form, there are from 17 to 21 spots, which are more or less rounded. On the prothorax there are usually from 4 to 6 spots, and there is also one sutural spot behind the scutellum. The small marginal spot is sometimes lacking, especially in western forms. The lower surface is black, with the exception of the head, margins of the prothorax, and that portion of the elytra which can be seen from below. The antennæ and legs are also black.

This form can readily be distinguished from all other genera of tortoise beetles by the characters given, and from other common species that attack sweet potato it can be known at once by its much larger size, since it is one of the largest of the leaf-beetle family occurring in the United States.

The full length is about one-third of an inch (8-11 millimeters) and the width about one-fourth of an inch (6-7 millimeters).

Several forms of this species occur in the United States which are considered to be merely varieties, races, and, in one instance, a subspecies. So far as known, these variants have practically the same habits, but some have a different distribution and all very closely resemble the form described above.

#### SYNONYMS AND VARIETIES

*Chelymorpha cassidea* Fabricius, Syst. Ent., 1775, p. 82.

*Chelymorpha argus* Lichtenstein, Cat. Mus. Hamb., 1795, p. 66.

*Chelymorpha cribraria* Olivier, In Enc. Meth., v. 5, 1790, p. 383; Ent., v. 6, 1808, p. 956.

Var. *lewisi* Crotch, in Proc. Acad. Nat. Sci. Phila., 1873, p. 77.

Var. *phytophagica* Crotch, l. c., p. 77.

Var. *sepiendecim-punctata* Say, in Jour. Acad. Nat. Sci. Phila., v. 3, 1823, p. 435.

Subsp. *geniculata* Bohemann, Mon., v. 2, 1854, p. 39.

#### EGG

The egg mass (fig. 2) of *Chelymorpha cassidea* is most peculiar. The eggs are deposited normally on the lower surfaces of the leaves and probably elsewhere at times, since in confinement masses have been found on the stalks. In the field they are deposited in clusters varying from 16 to 28. Six masses contained 16, 17, 18, 24, 26, and 28 eggs, respectively. The eggs are attached to the leaf by long pedicels, the pedicels being fastened to the leaf surface by a considerable amount of glutinous substance. The eggs also adhere to each other at their bases and, in many cases, halfway or a little more toward the apices, but the ends are free and divergent. The eggs are deposited irregularly, without definite pattern. There is usually a central irregular row of 6 eggs, flanked at each side by a similar irregular row of 5 or 6, while the outer rows together form a mass, which is always irregular, but with a suggestion of a circular arrangement.

The individual egg measures about 1.6 millimeters in length and 0.8 millimeter in width, being approximately twice as long as wide. It is evenly rounded at the base and bears at the distal end a cap which opens at one side when the larva issues and which bears at the extreme apex a prominent dark reddish tubercle of irregular shape, somewhat resembling a bit of sealing wax. The general color of the egg is dull buff and the surface is granulated.



## LARVA

The larva (fig. 1, *b*; Pl. 1, A, *b*; B) may be described as follows:

Head prominent, dark brown, outline of basal half semicircular; eyes small, black; mandibles prominent, darker brown, width of head about 1.6 millimeters; surface with numerous short bristles. Thoracic plate nearly twice as long as wide, each half irregularly pentagonal. Body dull light yellow, strongly marked with numerous dark brown, nearly tuberculate spots; its form, including thorax, robust, less than twice as long as wide, somewhat depressed, armed with long prominent lateral spines, 14 on each side as follows: 4 thoracic, first pair directed forward and upward above head, second pair at acute angles and semierect and two pairs at right angles; 8 abdominal spines, slightly curving upward at apex, 2 anal spines erect. Each spine wide at base and light colored in basal half with strong lateral spines, apical half acuminate, black. Dorsum of abdominal segments each with two rows of transversely rounded oblong tubercles, those of first three segments largest, size diminishing posteriorly. Anal segment with well defined dark brown plate about twice as long as wide, terminating in a long proleg rounded at apex. The feci-fork is usually held slanting back from the body and rarely vertical or over it.

Lower surface with a median row of 5 small, rounded, longitudinal brown spots on segments 2 to 6, 6 to last with transverse dark-brown marks, growing stronger to last segment, posterior third more or less marked with dark brown. Legs long and stout, dark brown, blackish when folded.

Length of full-grown larva without feci-fork 7 millimeters (about  $\frac{1}{2}$  inch), width 4.5 millimeters.

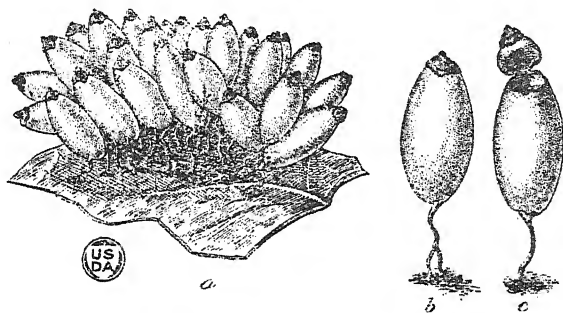


FIG. 2.—Eggs of the argus tortoise beetle: *a*, Mass of eggs, attached to leaf; *b*, *c*, individual eggs before and after hatching.

## PUPA

The pupa (fig. 1, *c*, *d*; Pl. 1, A, *c*) is pale yellow, marked with dark brown, becoming nearly black toward the time of transformation to adult. The surface is nearly covered with a pale bluish bloom or waxy secretion resembling a mold, a peculiar covering not often seen in any other group of beetles. The ventral surface is somewhat flattened and the dorsal surface is convex. The thorax projects strongly at each side, being a little wider than the widest abdominal segment. It is armed apically with two short spinous processes in the proximal third. The antennal sheaths and legs are robust, the posterior pair being about as long as the elytral sheaths. The body is armed on each side with five long and strong black-tipped spines similar to those of the larva, the first pair situated about the middle of the body. The first two pairs are subequal in length and the remaining pairs decrease in length posteriorly. There are also two pairs of short unicolorous spines toward the apex. The cast skin of the larva with its spines is rolled up in a mass at the posterior extremity.

Length 8 millimeters, width 4.5 millimeters.

## DISTRIBUTION

The Argus tortoise beetle is a native species inhabiting a large portion of the United States. It also occurs in Canada. The species does not appear to be recorded from Mexico, but several related species occur there. The known distribution, including what at present are considered varieties, is shown in Figure 3.

## NOTES ON HABITS AND DEVELOPMENT

From larvae collected at Arlington, Va., June 20, 1919, and later, the first adults began to emerge June 29, continuing to emerge until July 3. In outdoor rearing cages emergence was from September 10 to the first week of October. Adults observed on sweet potato all developed within 7 to 10 days of each other. Individuals collected by Miss Marion T. Van Horn on wild bindweed (*Convolvulus* sp.) that grew in a shady location in the District of Columbia were, evidently as a result of not being exposed to direct sunlight, over two weeks late in development, while larvae one-third to two-thirds grown were observed after the Arlington material, which was almost constantly exposed to sunlight, had all transformed to pupæ.

In 1919, 50 reared beetles were under observation during July. Of this number 20 were placed on growing sweet potato plants and covered

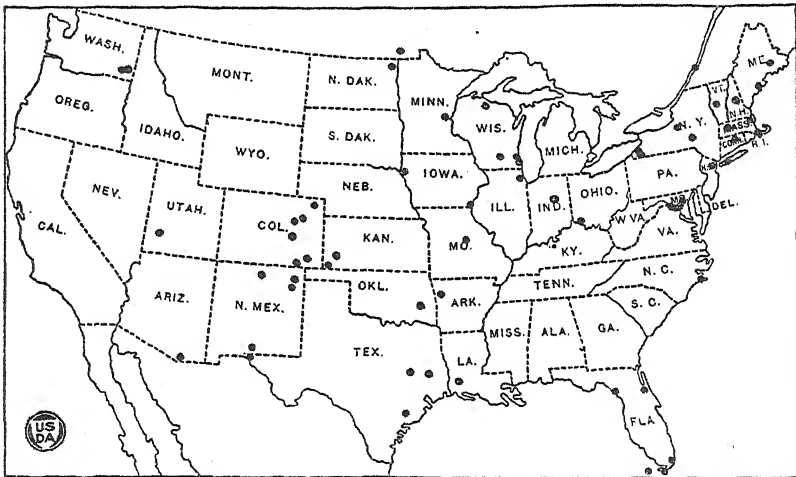


FIG. 3.—Map showing known distribution of the Argus tortoise beetle.

with a large cloth-covered rearing cage, but they did not thrive, some individuals dying, and no eggs could be found up to the end of the month, although in a second lot kept in the insectary eggs were observed August 3. Finally the cage was removed and the beetles allowed to shift for themselves. September 2 an egg mass was found on the same plat where the beetles had been feeding.

The experience of two years shows definitely that the second generation is only a partial one, since only three egg masses of this generation were found and at intervals of a month, indicating that the majority of the beetles of the first new generation hibernate, in this respect agreeing with some other insects.

Of the second generation, the eggs of which hatched during August, several pupæ were formed a month later, showing a larval period of about three weeks in rather cool summer weather.

In its apparently irregular development, *Chelymorphism cassidea* resembles to some extent the Colorado potato beetle. The overwintered beetles first occur some time in May—in 1920, May 17, in an exception-

ally cool spring. The first egg masses were obtained May 23. The first new generation develops during the last week of June and throughout July, with the temperature 60° to 100° F., averaging 75°.

May 17, 1920, adults were collected on the western edge of the truck farm at Arlington, Va., and on the far side of a large sewer pipe which had undoubtedly attracted them, as it conserved considerable heat. Nearly all of these beetles were on the west side, morning-glory plants which were numerous on the east side harboring only one specimen. The beetles hibernated on the more protected side and mostly together, since all specimens were found in two small areas, representing colonies, quite close to each other. Additional evidence of the eminently gregarious habit of this species was afforded the following morning by examination of the jar in which these beetles were placed overnight. At first glance it was thought that some had escaped, but close examination showed that they were closely huddled together in the folds of the small leaves.

Larvæ obtained in late August transformed to pupæ September 1, and the adults emerged September 10, which gives 9 days for the pupal period during moderately cool weather, with the temperature ranging from 70° to 82° F., and averaging 74° F.

The first pupa was observed on June 23. The larval period is about three weeks in cool summer weather, and the pupal period for the same temperature is about nine days. The fact that the species has only an exceptional second generation in the District of Columbia tends to show that it is single-brooded in the North and fully double-brooded in the Southern States.

The adults issue at any time during the day, and the coloring begins at the head and legs, the dots on the prothorax appearing some time before those on the elytra. When first emerged the beetles are bright yellow, afterwards changing to a darker yellow and finally to yellowish red or dark brick red. For full coloring the insect requires at least two, and probably three days.

The beetles cling most tenaciously to foliage or to objects in rearing cages, unless they drop down to "play 'possum," and evidently for that reason do not very often find their way into the collecting net, most individuals having been collected on their food plants and elsewhere, where attention was attracted to them because of their conspicuous coloring.

The first eggs obtained during 1920 were laid during the last week of May, beginning May 22, and the first larvæ were noticed June 10. Eggs that were laid June 4 and were isolated, hatched June 14, 10 days later. From this lot the larvæ began to transform to pupæ July 3 and the adults began issuing July 10. The pupæ began to transform to beetles at the same date in different jars kept under different atmospheric conditions.

The foregoing data furnish the following as an approximate average life cycle from egg to adult for the District of Columbia and vicinity:

PERIODS OF THE STAGES OF CHELYMORPHA CASSIDEA FAB.

Egg period:	Days.
June 1 to June 11.....	10
June 4 to June 14.....	10
Larval period: June 14 to July 3.....	19
Pupal period: July 3 to July 10, September 1 to 10.....	7 to 9
Total from egg to adult.....	36

## HISTORY AND LITERATURE

The Argus tortoise beetle was described, under the name *Coccinella cassidea*, by Fabricius in 1775 (3<sup>3</sup>, p. 82).

In 1869, A. S. Packard (10, p. 504) figured the pupa and adult, with the statement that he had found all stages on the leaves of "silkworm" in July and early in August, and that in one instance in Salem, Mass., it occurred in abundance on raspberry.

In 1870, Riley (11, p. 58; 12, p. 4) published a short note on the larva and adult, stating that it was found on *Asclepias*.

In 1879, Harrington (2, p. 120) published a short note in which the species was reported as a new foe by market gardeners in Canada, where it occurred in immense numbers and destroyed plants and flowers. It was said to be first noticed on wild *Convolvulus*.

In the early eighties the writer received from Ovid, N. Y., specimens of the larva of the Argus tortoise beetle on raspberry.

The first record of the Bureau of Entomology is dated March 11, 1884, when specimens were received which were taken on the foliage of sweet potato at Touch Key, Fla.

June 28, 1886, Dr. J. M. Shaffer, Keokuk, Iowa, reported numbers of larvæ on sweet potato vines.

In 1887, Lintner (8, p. 673) wrote of this insect under the title "Milkweed beetle with bad habits." A correspondent in Chenango County, N. Y., stated that it was found on morning-glory, corn, cabbage, and plantain. In Doctor Lintner's reply he mentions other alleged food plants, including milkweed, mustard, and plants of the rose family.

In 1889, J. B. Wielandy reported the species on *Convolvulus* at Springer, N. Mex., on July 13, and on June 30 attack on sweet potato was reported at Slatonville, Ark., by D. D. Forman.

In 1893, Webster (14, p. 204) treated this insect as occurring on raspberry and blackberry.

In 1897, the writer published what appears to be the first account of attack by this species on sweet potato (2, p. 23). In 1898, Webster and Mally (15, p. 99) mentioned its occurrence at Willard, Ohio, on strawberry vines. In 1899 Lugger (9, p. 254) mentioned this insect as frequently being found on raspberry. In 1899, also, Sanderson (13, p. 140-142) gave a biological account of this species, with breeding records showing variability in the adults from the same mass of eggs. He also described the eggs, larvæ, and pupæ.

July 31, 1901, specimens were received from J. P. Reynolds, North Haven, Me., taken on rose leaves.

In 1905, Forbes (5, p. 192) included this species in a list of insects found on corn.

July 8, 1906, specimens were received from Silver Creek, N. Y., found on timothy, evidently an accidental occurrence. The same comment applies to specimens found next year in a cornfield at Arlington, Va. July 19, 1907, larvæ were reported by I. J. Condit attacking sweet potato at Benning, D. C., and in that year Fall and Cockerell (4, p. 200) mentioned its occurrence on *Solanum* in New Mexico.

August 15, 1908, Otis Andrews, El Paso, Tex., reported injury to morning-glory and moonflower. July 22, 1909, a canning company at Cherry Creek, N. Y., sent specimens of pea vines on which were found

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 50-51.

many larvæ and adults of this tortoise beetle. The vines were also well covered with pupæ from which the adults were beginning to issue. July 28, Miss Julia D. Whiting, Deerfield, Mass., sent specimens found on morning-glory, including larvæ with the nymphs of the pentatomid predacious enemy *Apateticus bracteatus* Fitch. The same year Frederick Knab (7, p. 152) wrote a short note on the nuptial colors of this species.

July 15, 1910, report was received from F. H. Horsford that the pupa was found on the lower side of the leaves of *Lilium henryi* at Charlotte, Vt.

July 13, 1911, Fabian Garcia, Agricultural College, N. Mex., reported attack on sweet potato. July 2, 1913, F. B. Milliken collected this species at Garden City, Kans., on bush morning-glory (*Ipomoea leptophylla*).

In 1916, H. S. Barber (1, p. 119) included this species in a review of North American tortoise beetles, summing up briefly the habits of the species as published and furnishing a map showing its distribution.

May 16, 1917, J. A. Hanchey, Allen Parish, La., reported attack on sweet potato, the plants looking as though fire had gone through them. This, however, was only in spots and did not extend through entire fields.

During 1919 this insect, as previously stated, attracted much attention. It was first noticed attacking sweet potato at Arlington, Va., when full grown larvæ were observed. June 28, 1920, a farm hand at Arlington, Va., noticed it on sweet potato and expressed the usual apprehension of injury.

July 21, 1922, Prof. H. F. Wilson, Madison, Wis., reported this species as being very common in Wisconsin and as creating considerable apprehension. Later he wrote that reports of damage were obviously erroneous and that the insect was not in reality a pest in that State.

#### NATURAL ENEMIES

The United States Bureau of Entomology has records of three natural enemies of this tortoise beetle; an egg parasite, a larval parasite, and a predacious bug.

*Emersonella niveipes* Girault.<sup>4</sup>—From egg masses collected by Miss Van Horn in the District of Columbia June 28, 1919, *Emersonella niveipes* Gir., a minute chalcidoid, began emerging July 12, 1919. From eggs collected by the writer September 5, 13 of these parasites emerged from one egg mass consisting of 19 eggs, in each case issuing from a round hole on one side near the top of the egg.

*Masicera exilis* Coquillett.—During July of 1907 several mature larvæ were observed in the District of Columbia on sweet potato leaves, from which the tachinid fly *Masicera exilis* issued July 19 to 29. July 2, 1919, the same species was reared from a larva from Arlington, Va.

*Apateticus bracteatus* Fitch.—July 26, 1909, Miss Julia D. Whiting, Deerfield, Mass., sent specimens of a large pentatomid bug, *Apateticus bracteatus* (fig. 4), which were observed attacking the larva of the Argus tortoise beetle in that vicinity. The nymphs transformed to adults August 4 to 7.

<sup>4</sup> Determined by A. B. Gahan.

The Biological Survey has found the Argus tortoise beetle in the stomachs of 14 species of birds, most often in those of the starling (*Sturnus vulgaris*) and kingbird (*Tyrannus tyrannus*).

#### GENERAL SUMMARY

The foliage of sweet potato, bindweed, and morning-glory is attacked by the adults and larvæ of the Argus tortoise beetle (*Chelymorpha cassidea*). Reports of attack to plants other than Convolvulaceae are in the main, if not entirely, erroneous.

The species has been studied in the District of Columbia. The eggs are deposited in clusters, varying from 16 to 28, on the lower surface of the leaves. They hatch in about 10 days into light yellow larvæ, which

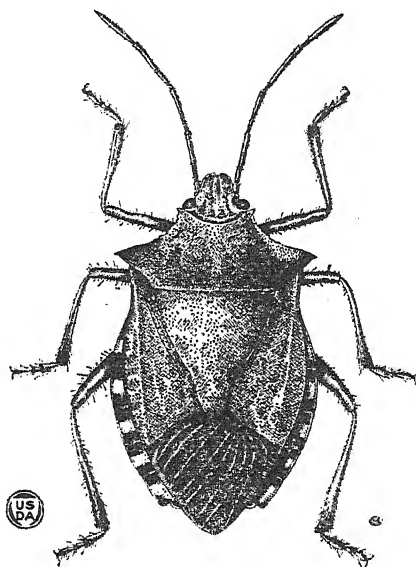


FIG. 4.—*Apateticus bracteatus*, a predacious bug enemy of the Argus tortoise beetle.

are gregarious and feed on the lower side of the foliage. In about three weeks they become mature and develop into similarly colored pupæ, which in from 7 to 9 days give forth the beetle. The species is evidently single-brooded in the North, double-brooded southward, and in the District of Columbia there is an exceptionally small second generation.

The Argus tortoise beetle is seldom so abundant as to be very destructive, plants readily recovering from its attack. The insect may be hand-picked in all stages and larvæ and adults can be killed with arsenicals.

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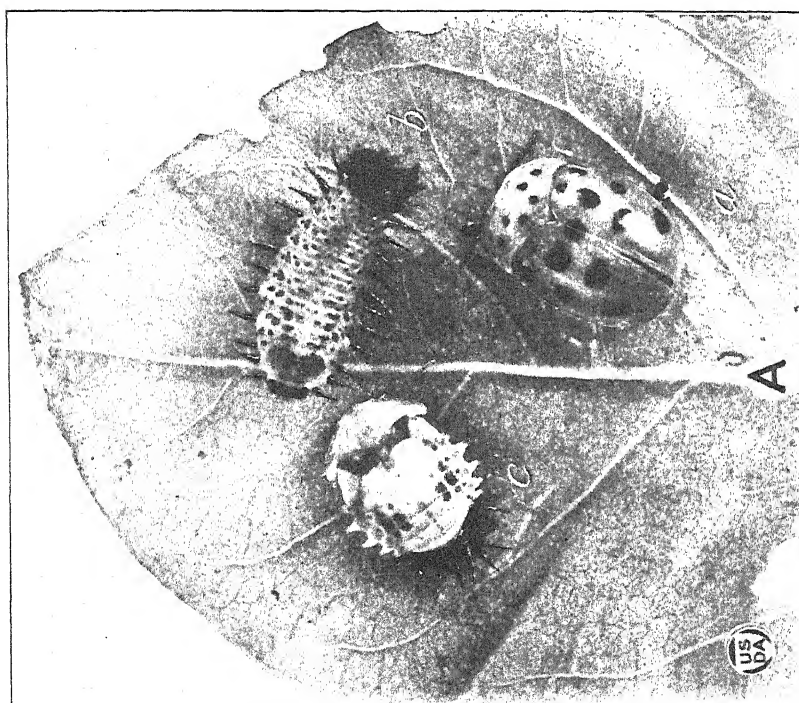
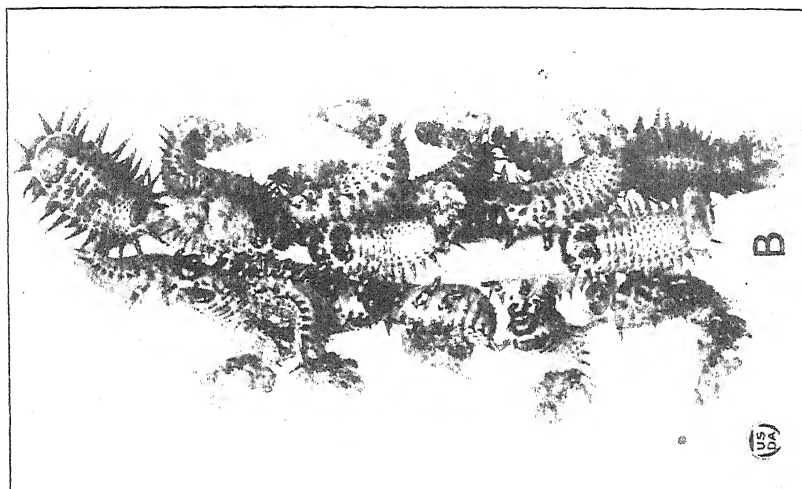
PLATE I

*Chelymorpha cassidea*

A.—Sweet-potato leaf showing (a) adult, (b) larva, and (c) pupa.

B.—Half-grown larvæ preparing to molt at tip of stem on which they have devoured the foliage.







# SEED-COLOR INHERITANCE IN CERTAIN GRAIN-SORGHUM CROSSES<sup>1</sup>

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## INTRODUCTION

Color of seed is one of the most evident varietal characters of the grain sorghums. Though it is not of major economic importance, yet it does influence the popularity of a variety. On the Kansas City and Wichita markets yellow milo is generally quoted at 10 cents per 100 pounds higher than white kafir. On the other hand, brown-seeded sorghums do not command as high a price or sell as readily as do the varieties with white, yellow, or red seeds.

Naturally, color of seed has attracted the attention of the plant breeder working with sorghums as it has that of workers with most other cereal crops. Graham (2)<sup>2</sup> of India investigated the inheritance of seed color in natural and artificial sorghum crosses. The colors studied were red, yellow, and white. Red was found to be dominant to yellow and white, and yellow dominant to white. In a cross between a yellow- and a white-seeded sorghum, the heterozygote was red seeded, segregating in the F<sub>2</sub> generation in a ratio of 9 red : 3 yellow : 4 white. Karper and Conner (6) and Sieglinger (8) determined the amount of cross-pollination in milo by taking advantage of the fact that the seed of the F<sub>1</sub> of a cross between white and yellow milo is yellow. Vinall and Cron (9), in reporting the inheritance of certain sorghum characters, state that a close agreement to a 9:7 dihybrid ratio for brown and white seed was obtained in crosses between Blackhull kafir and feterita. This color inheritance was attributed to two factors—*B*, a factor for brown color, carried by the Blackhull kafir, and *S*, a spreader factor carried by the feterita.

Conner and Karper (1), in a paper published after the original draft of this paper was written, reported on the inheritance of seed-coat color in the hybrids Dwarf Yellow × Dwarf White milo, Blackhull × Red kafir, and Blackhull × Pink kafir. They found the F<sub>1</sub> in the milo cross to be indistinguishable in color from the yellow parent, the F<sub>2</sub> segregating in the ratio of 3 yellow to 1 white. In the Blackhull kafir × Red kafir cross, the seeds borne by the F<sub>1</sub> plants were pale red, intermediate in color between those of the parents. The F<sub>2</sub> segregated in the ratio of 1 white, 2 pale red, and 1 red. The phenotypic classification of the material was substantiated by growing the F<sub>3</sub>, practically all of the whites and reds remaining constant and the pale reds segregating. In the cross Blackhull kafir × Pink kafir the F<sub>1</sub> was intermediate in seed color between the parents, the F<sub>2</sub> segregating in the ratio of 1 white, 2 pale pink, and 1 pink. No hybrid vigor was noted in any of these three crosses between sorghum varieties, the parents apparently being similar in most respects except seed color.

<sup>1</sup> Accepted for publication November 19, 1923.

<sup>2</sup> Reference is made by numbers (*italic*) to "Literature cited," p. 64.

## EXPERIMENTAL MATERIAL AND METHODS

Several crosses between different varieties of grain sorghums were made by the writer at the Woodward Field Station, Woodward, Okla., in 1919 and 1920. Some of these have been grown through the  $F_3$  generation, while the remainder have been carried through the  $F_2$  generation. The crosses discussed in this paper are (1) *feterita*  $\times$  *Sunrise kafir* and its reciprocal, (2) *Sunrise kafir*  $\times$  *Blackhull kaoliang*, (3) *feterita*  $\times$  *Red kafir*, (4) *Sunrise kafir*  $\times$  *Red kafir*, (5) *White kafir*  $\times$  *Red kafir*, and (6) *White kafir*  $\times$  *Sunrise kafir*.

## SEED COLOR OF PARENT VARIETIES

*Feterita* kernels are chalky or starchy white in external appearance, but portions of the seeds frequently are stained a dark red. This discoloration is most apparent on that part of the kernel covered by the glumes. The kernels of the selection of *Blackhull kaoliang* used in this study have the same sublenticular shape and peculiar chalky white color as those of *feterita*. It was selected from the progeny of bulk seed of *Barchet kaoliang* obtained in 1915 at the Amarillo Cereal Field Station. From the shape and color of the seeds, it is believed to have arisen through natural crossing between this *kaoliang* and *feterita*.

*Sunrise kafir* has the typical seed color of the white-seeded kafirs, creamy white with a more glossy surface than that of *feterita* and the *Blackhull kaoliang* hybrid. White kernels of *kafir* usually are marked with reddish black spots which occur most often at the point of attachment of the style. These spots vary in size and seem to be influenced to some extent by the environmental conditions that obtain during kernel development.

*Red kafir* kernels are dark brownish orange or brownish red with a smooth or glossy appearance. Frequently the color is not uniform over the kernel, due to bleaching where exposed to the weather.

Kernels of *feterita* and *Blackhull kaoliang* possess a layer of comparatively thick brown cells directly outside of the aleurone layer. This layer of cells is designated as the nucellar or hyalin layer, by Winton (10), the seed coat (*Samenschale*) by Harz (5, p. 1249-1254), and hyalin layer (*Hyalinschichte*) by Mitlacher (7), and probably is analogous to the inner integument in *Johnson grass* as described by Harrington and Crocker (3). Whatever this layer occurring directly outside of the aleurone layer may be called, it consists of relatively large cells containing a dark brown pigment. In *Sunrise* and other white kafirs, and in *Red kafir*, this layer of dark brown cells is absent. Winton (10) made a microscopic examination of the seeds of 11 varieties of sorghum. Of these, a nucellar layer was found in three varieties of broomcorn, *Early Amber* and *Early Orange* sorghos and *Brown durra*. The other varieties, *White* and *Red kafir*, *White* and *Yellow milo*, and *White durra*, had no nucellar layer. Winton's studies and the writer's examination of kernels of many sorghum varieties indicate that brown seed color is an indication of a brown nucellar layer. *Feterita*, *Blackhull kaoliang*, and *Dwarf hegari* have white seeds with a brown nucellar layer. A casual examination shows that these three white-seeded varieties which have a nucellar layer are chalky or starchy white in color, differing in this respect from many other white-seeded sorghums.

The writer is indebted to Dr. Carleton R. Ball for examining the seeds of several different introductions of white-seeded kaoliangs from China. He determines that two closely related varieties having black glumes and white seeds, namely, Barchet (C. I. 310, S. P. I. 22912) and Korean Blackhull (C. I. 412, S. P. I. 27553) both possess a brown nucellar layer like that of *feterita*. On the other hand, Brill Blackhull (C. I. 120, S. P. I. 17920), Mukden White (C. I. 190 and 272, S. P. I. 18610 and 21077), and an unclassified white-seeded variety (C. I. 350, S. P. I. 29166) show no trace of a brown nucellar layer. He reports also that the seeds of the varieties having the brown layer are whiter and more chalky in appearance than those not showing it, thus resembling *feterita* seeds, while the others more nearly resemble *kafir* seeds.

## EXPERIMENTAL RESULTS

SUNRISE KAFIR  $\times$  FETERITA AND THE RECIPROCAL CROSS

Reciprocal crosses were made between Sunrise *kafir* and *feterita* in 1919. A number of crossed seeds were obtained, all of which resembled the pistillate parent, there being no indication of *xenia*. Some of these seeds were sown in 1920, and several of the resulting  $F_1$  heads were selfed. The seed produced on the  $F_1$  plants was tan or light brown, with a brown nucellar layer similar to that of *feterita*. As neither of the parents had brown seeds, the production of brown seeds in the  $F_1$  was not expected.

TABLE I.—Distribution of seed-color in the  $F_2$  generation of the cross Sunrise *kafir*  $\times$  *feterita*, and its reciprocal

Designation.	Plants.				
	Year.	Total number.	Having seeds with—		
			Brown nucellar layer present.		Brown nucellar layer absent, seeds white.
			Seeds brown.	Seeds white.	
Sunrise <i>kafir</i> $\times$ <i>feterita</i> .....	1921	179	111	29	39
Do.....	1921	145	85	26	34
<i>Feterita</i> $\times$ Sunrise <i>kafir</i> .....	1921	193	111	33	49
Sunrise <i>kafir</i> $\times$ <i>feterita</i> .....	1922	156	100	24	32
<i>Feterita</i> $\times$ Sunrise <i>kafir</i> .....	1922	172	93	31	48
Do.....	1922	174	101	34	39
Do.....	1922	163	97	34	32
Total.....		1,182	698	211	273
Calculated (9:3:4 ratio).....			664.9	221.6	295.5
Deviation.....			33.1	10.6	22.5

Seeds from two of the  $F_1$  heads of Sunrise *kafir*  $\times$  *feterita* and one head of the reciprocal cross were sown in head rows in 1921. Preliminary counts of the  $F_2$  heads in the plat showed clearly that the seed-color character was influenced by more than one factor. When mature, one head was harvested from each plant for a study of seed color. The  $F_2$  heads of these crosses were readily placed in three groups according

to seed color—(1) brown or light brown, with a brown nucellar layer; (2) chalky or starchy white, with a brown nucellar layer, similar to the *feterita* parent; and (3) creamy white with no brown nucellar layer, similar to the *Sunrise kafir* parent. To determine the presence or absence of the brown nucellar layer, a kernel was taken from each head and the outer coat scraped off with a knife. This method of determination proved to be accurate and satisfactory.

Some of the crossed seeds obtained in 1919 were not sown until 1921, when several  $F_1$  heads were selfed. One head row of  $F_2$  plants of *Sunrise kafir*  $\times$  *feterita* and three head rows of the reciprocal cross were grown in 1922. This  $F_2$  material was similar to that obtained in the preceding year. Data on the distribution of seed color in the  $F_2$  of this cross and its reciprocal are shown in Table I.

Using the method of Harris (4) for testing the goodness of fit of Mendelian ratios,  $\chi^2 = 3.86$  and  $p = .1476$ , or a variation of the size obtained might be expected once in seven cases, due to random sampling. It appears that the segregation of color factors in a *Sunrise kafir*  $\times$  *feterita* cross was according to the dihybrid ratio of 9:3:4. To explain this inheritance the following factors may be assumed:

*B*, a factor for brown nucellar layer which also may cause brown in the epidermis if *S* is present. Its allelomorph *b*, gives kernels without a brown nucellar layer.

*S*, a factor for smooth or glossy pericarp. When *S* is present the pericarp is glossy and may be creamy white, as in white kafir, or may carry other colors. Its allelomorph *s*, gives a chalky white pericarp.

Brown does not appear in the pericarp of an *ss* plant.

The parents and  $F_1$  hybrids, so far as shown by the results of this cross, then would have the following factorial composition:

*Feterita*..... *BBss*.

*Kafir*..... *bbSS*.

$F_1$  hybrid..... *BbSs*.

The  $F_1$  kernels having one *B* factor have a brown nucellar layer. As an *S* factor also is present, the brown color appears in the epidermis. The  $F_2$  results seem to substantiate this hypothesis. The results expected in the  $F_2$  generation, from an independent recombination of the two factors just designated, would produce three phenotypes classified as follows:

Brown seeded, with brown nucellar layer.	Chalky white seeded, with brown nucellar layer.	Glossy white seeded, with no brown nucellar layer.
1 <i>BBSS</i>	1 <i>BBss</i>	1 <i>bbSS</i>
2 <i>BBss</i>	2 <i>Bbss</i>	2 <i>bbSs</i>
2 <i>BbSS</i>	—	1 <i>bbss</i>
4 <i>BbSs</i>	3	—
—		4
9		

The data presented in Table I are in substantial agreement with the hypothesis here presented. The *bbss* seeds with a chalky white color, but with no brown nucellar layer, because of weather effects, are difficult to distinguish when this chalky white is not over a brown nucellar layer,

and were classified with the glossy white phenotype with no nucellar layer.

A number of selfed heads were obtained from the  $F_2$  rows of Sunrise kafir  $\times$  feterita and its reciprocal grown in 1921. Seed from these and from several unbagged heads were sown in head rows in 1922 to determine the behavior of the material in the  $F_3$  generation. Descriptions of the seed from the  $F_2$  heads and the phenotypic distribution of the  $F_3$  progenies grown from them in 1922 are shown in Table II.

TABLE II.—Description of  $F_2$  seed heads of the cross, Sunrise kafir  $\times$  feterita, and its reciprocal, and distribution of the progenies obtained from these heads into seed-color classes in 1922

Description of seed heads.	Distribution of F <sub>2</sub> progeny.			Factorial composition.
	Brown nucellar layer present.		Brown nucellar layer absent, white seeds.	
	Brown seeds.	Chalky white seeds.		
Sunrise kafir × feterita (row 1 in 1921):				
1. Not bagged, white, no nucellar layer...	0	0	40	{bbss, or bbSs, bbSS
2. Not bagged, brown, nucellar layer.....	86	27	0	BBsS
3. Selfed, brown, brown nucellar layer...	57	25	22	BbSs
4. Do.....	124	0	0	BBSS
5. Not bagged, white, brown nucellar layer	0	78	23	Bbss
Sunrise kafir × feterita (row 2 in 1921):				
1. Selfed, brown, brown nucellar layer...	74	0	27	BbSS
2. Do.....	113	43	48	BbSs
3. Do.....	82	30	40	BbSs
4. Not bagged, white, no nucellar layer...	0	0	123	{bbss, or bbSs, bbSS
5. Selfed, brown, brown nucellar layer....	60	23	34	BbSs
6. Do.....	114	0	33	BbSS
7. Selfed, white, brown nucellar layer....	0	148	0	BBss
Feterita × Sunrise kafir:				
1. Selfed, white, brown nucellar layer....	0	90	26	Bbss
2. Do.....	0	79	29	Bbss
3. Do.....	0	89	0	BBss
4. Selfed, white, no nucellar layer.....	0	0	133	{bbss, or bbSs, bbSS
5. Selfed, white, brown nucellar layer....	0	94	0	BBss
6. Selfed, brown, brown nucellar layer....	85	31	0	BBSS

The factorial constitution of most of the  $F_2$  heads used as seed can be determined definitely from the  $F_3$  results. This is given in the last column of Table II, on the basis of the two-factor hypothesis previously outlined, and will account for all observed types. These two factors, therefore, not only account for the surface color of the kernel, but also for the presence or absence of the very apparent brown nucellar layer.

The ratios obtained in the  $F_3$  generation, while not exact, are as close to the expected as such small numbers of plants would require. Three possible factor combinations are suggested for the parent heads of the

three pure white  $F_3$  progenies. Any of the factor combinations indicated for these  $F_2$  heads would produce all white-seeded plants, without brown nucellar layer, in the  $F_3$  generation, and consequently the specific one can not be determined.

#### SUNRISE KAFIR $\times$ BLACKHULL KAOLIANG

A cross was made in 1919 between Sunrise kafir and Blackhull kaoliang, using Sunrise kafir as the pistillate parent. Reciprocal crosses were attempted, but the Blackhull kaoliang spikelets seemed to be very susceptible to mutilation and the emasculated flowers dried without producing seed. Part of the crossed seeds were sown in 1920 and the remainder in 1921.

The  $F_1$  kernels of the Sunrise kafir  $\times$  Blackhull kaoliang cross were of a tan or brown surface color. A brown nucellar layer also was present. The similarity in appearance of feterita and Blackhull kaoliang kernels and the similarity in the color of the seeds of  $F_1$  crosses between these varieties and Sunrise kafir indicate that the same or very similar color factors are present in feterita and Blackhull kaoliang. As previously stated, the strain of kaoliang used probably is a selection from a natural hybrid between Barchet kaoliang and feterita.

Seed from two selfed heads of the  $F_1$  generation grown in 1920 were sown in head rows in 1921, and two similar head rows were grown in 1922 from  $F_1$  plants selfed in 1921. The data on seed color obtained from the  $F_2$  progenies in 1921 and 1922 are presented in Table III.

TABLE III.—*Distribution of seed color in the  $F_2$  generation of the cross Sunrise kafir  $\times$  Blackhull kaoliang, grown in 1921 and 1922*

Row number.	Year.	Plants.			
		Total number.	With seeds having—		
			Brown nucellar layer present.		Brown nucellar layer absent, seeds white.
			Seeds brown.	Seeds white.	
1.....	1921	224	132	44	48
2.....	1921	226	132	47	47
1.....	1922	227	133	46	48
2.....	1922	250	159	43	48
Total.....		927	556	180	191
Calculated (9:3:4 ratio).....			521	174	232
Deviation.....			35	6	41

$\chi^2=9.803$ .  $P=.0076$ , 1 in 131, or 0.76 per cent.

The results indicate that the segregation of seed-color factors in the Sunrise kafir  $\times$  Blackhull kaoliang cross is similar to that in the crosses between Sunrise kafir and feterita. While the data in Table III diverge somewhat widely from the calculated ratios, yet in view of similar data from other crosses the agreement is close enough to confirm the two-factor hypothesis.



Seed of four selfed  $F_2$  heads was sown in head rows in 1922 and the data shown in Table IV were secured.

TABLE IV.—Description of  $F_2$  seed heads of the cross Sunrise kafir  $\times$  Blackhull kaoliang and distribution of the  $F_3$  progenies of these heads into seed-color classes in 1922

Description of F <sub>2</sub> seed heads.	Plants with seeds having—			Factorial composition.
	Brown nucellar layer present.		Brown nucellar layer absent, seeds white.	
	Seeds brown.	Seeds white.		
Selfed, brown, brown nucellar layer . . . . .	124	43	51	BbSs
Do. . . . .	103	35	46	BbSs
Selfed, white, brown nucellar layer . . . . .	0	64	11	Bbss
Do. . . . .	0	148	47	Bbss

The distribution of progeny from all four rows of  $F_2$  material in 1921 and 1922 differed considerably from the calculated 9:3:4 ratio, there being a surplus of brown-seeded plants with brown nucellar layer and a deficiency of white-seeded plants without brown nucellar layer. However, rows 1 and 2 of the  $F_3$  generation closely approach a perfect 9:3:4 distribution, the actual distribution of 402 plants being 227:78:97, as compared with a calculated distribution of 226:75:101.

From the data reported here on  $F_1$ ,  $F_2$ , and  $F_3$  material of crosses between Sunrise kafir and feterita and between Sunrise kafir and Blackhull kaoliang, it appears that the seed color of feterita and Blackhull kaoliang is due to the presence of two color factors designated as *BB* and *ss*, while the seed color of Sunrise kafir is due to two color factors designated as *bb* and *SS*.

#### FETERITA $\times$ RED KAFIR

In view of the unexpected occurrence of brown color in crosses having feterita or Blackhull kaoliang as a parent, some data on a cross between feterita and Red kafir are presented to further substantiate the hypothesis that feterita carries a factor which produces a brown color.

A cross was made in 1920 between feterita and Red kafir, feterita being the pistillate parent. The kernels in the  $F_1$  heads produced in 1921 are brown, though the intensity of the brown varied, depending on the extent of weathering. On that part of the seed covered by the glumes there was quite a tinge of red color.

Seed from four selfed  $F_1$  heads was sown in head rows in 1922. One head from each  $F_2$  plant was harvested and taken to the laboratory, where color of seed was recorded. Many variations and tints of color were present in the  $F_2$  material and it was necessary to make rather broad classes to obtain accuracy. The classes used were as follows:

Brown seeds, with brown nucellar layer.

White seeds, with brown nucellar layer.

Red and pink seeds, with no brown nucellar layer.

White seeds, with no brown nucellar layer.

All the  $F_2$  heads could be placed in one of the above color classes with a fair degree of accuracy. The results of this classification of the  $F_2$  progenies of the feterita  $\times$  Red kafir cross are shown in Table V.

TABLE V.—Distribution of seed color in the  $F_2$  generation of the cross feterita  $\times$  Red kafir, grown in 1922

Row number.	Plants.				
	Total number.	Having seeds with—			
		Brown nucellar layer present.		Brown nucellar layer absent.	
		Seeds, brown.	Seeds, white.	Seeds, red or pink.	Seeds, white.
1.....	165	114	7	33	11
2.....	138	103	7	20	8
3.....	174	126	9	26	13
4.....	121	87	6	22	6
Total.....	598	430	29	101	38
Calculated (45:3:12:4 ratio).....		421	28	112	37

$$\chi^2=1.335. \quad P=0.72468.$$

A study of the color distribution in the  $F_2$  generation of the cross between feterita and Red kafir indicates that the two color factors involved in the cross between feterita and White kafir are concerned, and that in addition a third color factor for red, introduced by the Red kafir parent, must also be considered. The color factors in feterita which are concerned in this cross may be designated as  $BBssrr$ , while those of Red kafir may be designated as  $bbSSRR$ ,  $R$  being the factor for red color, as present in Red kafir, and  $r$  its recessive allelomorph for absence of the red color. The  $F_1$  cross then would be  $BbSsRr$ . The probable effect on seed color of the  $B$ ,  $b$ , and  $S$ ,  $s$  factors was stated in the discussion of the previous crosses and the  $R$ ,  $r$ , factors will be considered more fully later. A study of the feterita  $\times$  Red kafir cross indicates that when the factor for brown nucellar layer is present with the factor for red,  $R$ , the color of the kernel is brown rather than red, and the phenotype then is brown.

On the basis of three color factors in 64 individuals of the  $F_2$  generation there should be 45 plants with brown seeds with brown nucellar layer, 3 plants with white seeds with brown nucellar layer, 12 plants with red or light red seeds and no brown nucellar layer, and 4 plants with white seeds without brown nucellar layer. It must be assumed that the presence of either the  $S$  or the  $R$  factor will cause the brown color to appear in the epidermis of the seed. The actual color distribution obtained in the four  $F_2$  rows, as compared with the theoretical results, is shown in Table V. A deviation of this magnitude might be expected to occur once in 1.38 cases as the result of random sampling.

The fit of the observed to calculated distribution of the color phenotypes is very close, in fact much better than usually would be expected, considering the relatively small number of individuals, 598. The con-

clusion seems justified that in the feterita  $\times$  Red kafir cross there are three factors influencing the color of seed, and that the three color factors are independently inherited, i. e., there is no evidence of linkage.

The possible combinations of three factor pairs, *Bb*, *Ss*, *Rr*, in the  $F_2$  generation are grouped under four phenotypes, as follows:

Brown surface, brown nucellar layer present.	White surface, brown nucellar layer present.	Red or pink surface, brown nucellar layer absent.	White surface, brown nucellar layer absent.
1 BBSSRR	1 BBssrr	1 bbSSRR	1 bbSSrr
2 BbSSRR	2 Bbssrr	2 bbSSRr	2 bbSsrr
2 BBSSRr	3	2 bbSsRR	1 bbssrr
4 BbSSRr		4 bbSsRr	4
2 BBsRRR		1 bbssRR	
4 BbSsRR		2 bbssRr	
1 BBSSrr		—	
2 BbSSrr		12	
4 BBsRRr			
8 BbSsRr			
2 BBsRRr			
4 BbSsrr			
1 BbssRR <sup>a</sup>			
2 BbssRR <sup>a</sup>			
2 BBssRr <sup>a</sup>			
4 BbssRr <sup>a</sup>			
45			

<sup>a</sup> Under the climatic conditions occurring in 1922, there was no evidence of a phenotype similar to feterita but carrying red in the pericarp. It was concluded, therefore, that an *R* factor with a *B* factor in the presence of *ss* gave a seed which was classified with the phenotype having brown surface color and brown nucellar layer. However, in 1923, the  $F_2$  material from the same source as in 1922 showed a number of plants whose seeds possessed a brown nucellar layer and had a white outer color marked with red. These doubtless are individuals having the factorial constitution of BBssRR, BbssRR, BBssRr, or BbssRr.

# SUNRISE KAFIR $\times$ RED KAFIR

In the discussion of the crosses previously described, in which feterita or Blackhull kaoliang was one parent and Sunrise or Red kafir the other parent, it was shown that brown seeds were obtained in the  $F_1$  generation and in more than half of the variates in the  $F_2$  generation.

The cross Sunrise kafir  $\times$  Red kafir was made in 1920. A few crossed seeds were sown in 1921, from which two selfed  $F_1$  heads were obtained. The seed from these was sown in head rows in 1922, and the segregation for seed color in the  $F_2$  generation of this cross is shown in Table VI.

TABLE VI.—Distribution of seed-color in the  $F_2$  generation of the cross Sunrise kafir  $\times$  Red kafir, grown in 1922

Row number.	Total number of plants.	Plants with red and tinted seed.	Plants with white seed.
1.....	144	111	33
2.....	143	101	42
Total.....	287	212	75
Expected (3:1 ratio).....		215	72

Deviation,  $3 \pm 4.95$ .  $\frac{\text{Dev.}}{\text{P. E.}} = 0.606$ .

The results shown in Table VI indicate that Sunrise kafir and Red kafir differ by a single main factor for seed color. The factors influencing color in the Sunrise  $\times$  Red kafir cross may be designated as *rr* for Sunrise kafir and *RR* for Red kafir. The seed color of the cross is due to the combination *Rr* in the  $F_1$  and the segregation of the *Rr* factor pair in the  $F_2$  generation in the monohybrid ratio of 3:1. While it seldom is advisable to formulate a factorial hypothesis on the behavior of only 287  $F_2$  individuals, in this instance it seems safe to do so, as the data on other crosses, White kafir  $\times$  Red kafir and White kafir  $\times$  Sunrise kafir, reported in this paper, bear out this theory. Sunrise kafir and White kafir have a similar seed color and the color results obtained in White kafir crosses should be the same as those in which Sunrise kafir is used.

#### WHITE KAFIR $\times$ RED KAFIR

A cross was made in 1919 between White kafir and Red kafir, the White kafir being the pistillate parent. Two  $F_2$  rows were produced in 1921 from seed of selfed  $F_1$  heads. The segregation of seed-color in the plants in these two head rows is shown in Table VII.

TABLE VII.—Distribution of seed color in the  $F_2$  generation of the cross White kafir  $\times$  Red kafir, grown in 1921

Row number.	Total number of plants.	Plants with red seeds.	Plants with white seeds.
1.....	202	150	52
2.....	198	151	47
Total.....	400	301	99
Expected (3:1 ratio).....		300	100

$$\text{Deviation, } \pm 5.84. \frac{\text{Dev.}}{\text{P. E.}} = 0.171.$$

Six selfed heads from each of the two  $F_2$  rows of this cross were used for sowing head rows in 1922. These 12 heads were selected for other characters than color of seed, the selection not being random, but seed color was recorded both for the parent heads and the  $F_3$  progenies. The data obtained in the  $F_3$  generation are shown in Table VIII.

Six of the  $F_2$  heads were classed as red and produced nothing but red-seeded progenies, with a total of 659 individuals. The three white-seeded  $F_2$  heads produced a total of 364 white-seeded individuals. The three parent heads classified as light red obviously were the heterozygotes, producing red- and white-seeded plants in a ratio fairly close to 3:1. As noted, the parent heads were not random selections, so that the fact that the progenies did not occur in a 1:2:1 ratio of dominants, heterozygotes, and recessives is immaterial. Especially is this true in view of the fact that the heterozygotes can be determined by their intermediacy.

TABLE VIII.—Distribution of seed-color in the  $F_3$  generation of the cross White kafir  $\times$  Red kafir in 1922

Designation.	Total number of plants.	Number of plants with—	
		Red seeds.	White seeds.
$F_2$ heads, red seeds:			
1-2.....	120	120	0
1-5.....	117	117	0
2-1.....	97	97	0
2-2.....	107	107	0
2-3.....	104	104	0
2-5.....	114	114	0
Total, 6 progenies.....	659	659	0
$F_2$ heads, white seeds:			
1-3.....	135	0	135
1-6.....	114	0	114
2-4.....	115	0	115
Total, 3 progenies.....	364	0	364
$F_2$ heads, light red seeds:			
1-1.....	117	85	32
1-4.....	110	81	29
2-6.....	110	81	29
Total, 3 progenies.....	337	247	90
Expected (3:1 ratio).....		253	84

Deviation,  $6 \pm 5.36$ .  $\frac{\text{Dev.}}{\text{P. E.}} = 1.119$ .

WHITE KAFIR  $\times$  SUNRISE KAFIR

A cross was made between White kafir and Sunrise kafir in 1919. The  $F_1$ ,  $F_2$ , and  $F_3$  plants grown from this cross produced kernels typical of white kafir in color, indicating that the genetic factors governing color of seed in these two varieties were the same.

## SUMMARY

The seeds of *feterita* and Blackhull kaoliang are a chalky opaque white. These varieties also have a well developed brown nucellar layer lying directly outside of the aleurone layer.

Seeds of Sunrise kafir are creamy white, with a smooth or glossy pericarp. No brown nucellar layer is present in the kernels of this variety.

Crosses between *feterita* or Blackhull kaoliang and Sunrise kafir produce  $F_1$  plants with light brown seeds with brown nucellar layer.

In the  $F_2$  generation of these crosses segregation occurs, the seed color of the three phenotypes being (a) brown seeds with brown nucellar layer, (b) white seeds with brown nucellar layer, and (c) white seeds with no brown nucellar layer. The ratio of these classes is approximately 9:3:4.

Results obtained in the  $F_3$  generation confirm the two-factor hypothesis of color inheritance in these crosses.

Red kafir seed is of a dark red color, with no brown nucellar layer. A cross between *feterita* and Red kafir produces a reddish-brown kernel with brown nucellar layer in the  $F_1$  generation.

In the  $F_2$  generation of the feterita  $\times$  Red kafir cross, four phenotypes are obtained, these being (a) brown with brown nucellar layer, (b) white with brown nucellus, (c) red and pink with no brown nucellus, and (d) white with no brown nucellus. The approximate ratio of these phenotypes is 45:3:12:4, indicating that three main factors influence the inheritance of seed color in this cross.

In the crosses Sunrise kafir  $\times$  Red kafir and White kafir  $\times$  Red kafir, segregation in the  $F_2$  generation occurs in a simple monohybrid ratio of three red-seeded plants to one white-seeded plant.

Crosses between White kafir and Sunrise kafir show that the seed-color of these two varieties is genetically identical.

The factors concerned in the inheritance of seed color in these sorghum crosses are *B*, a factor for brown nucellar layer, which produces brown color in the epidermis in the presence of *S*; *b*, its allelomorph, giving no brown nucellar layer when homozygous; *S*, a factor which will cause the development of color in the epidermis of the seed; *s*, its allelomorph, which, when present in the homozygous condition, will prevent the development of the brown color in the epidermis, *R*, a factor for dark red, which, when present, causes the development of red color in the epidermis; and *r*, its allelomorph, absence of red color.

The factorial constitution of feterita and Blackhull kaoliang with respect to seed color is *BBssrr*, of Sunrise and White kafir, *bbSSrr*, and of Red kafir, *bbSSRR*.

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# THE EUROPEAN CORN BORER, *PYRAUSTA NUBILALIS* HBN., VERSUS THE CORN EARWORM, *HELIOTHIS OBSOLETA* FAB.<sup>1</sup>

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The spread of the European corn borer, *Pyrausta nubilalis* Hbn., in the northeastern portion of the United States, during the last few years, creates interest in what will happen when this insect reaches the sections of the country where the corn earworm, *Heliothis obsoleta* Fab., seriously damages the corn crop. In the area infested by the European corn borer at present the corn earworm is present annually, but usually in small numbers, and seldom does serious injury to the corn.

In the late summer and fall of 1921 the corn earworm was abundant in late sweetcorn and in field corn in eastern Massachusetts. This was probably due to the occurrence of a second brood of the insect, which apparently was made possible either by the rather mild preceding winter or by the abnormally long growing season of that year. The infestation in some fields ran as high as 90 per cent of the ears, and injury was very apparent over a considerable area.

In the same season the European corn borer was also destructive in certain fields infested by the corn earworm, forecasting the condition which one might expect to find in the southern portion of the Corn Belt should the European corn borer ever reach that section.

Larvæ of both species were commonly found feeding in and on the same ears of corn, frequently their burrows uniting and adjoining, while the larvæ were in close proximity, each apparently too much occupied with the feast to be mindful of its neighbor.

Although not present in such large numbers, the larvæ of the corn earworm were able by the time they completed feeding to inflict more actual grain loss than the larvæ of the European corn borer, because of their greater feeding capacity and because their work was, for the most part, concentrated on the kernels, whereas the European corn borer larvæ feed throughout all parts of the ear and stalk, thus having a much wider range of activity. The feeding of the European corn borer larvæ, however, results frequently in more injury than is apparent in actual destruction of the grain, by injuring the stalk and particularly the peduncle of the ear. This often results in a reduction of the number of kernels forming on the ear. In 1921 many of the ears showed undeveloped kernels, particularly at the tips, which was undoubtedly due, at least in part, to the injury to other parts of the plants by the European corn borer.

A field of about an acre of Longfellow flint corn was used to obtain the information contained in this paper. The ears studied were selected at random and showed the average condition of the ears of the whole field. Ears shown in Plate 1 are average ears selected to show different types of injury rather than a maximum amount of damage.

When the counts shown in Table I were made (October 14, 1921) the corn earworm larvæ, in nearly all cases, were feeding in the ears. By October 20, 1921, when Table II was prepared, many of the larvæ of

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the corn earworm had deserted the ears to pupate. The injury, it will be observed, had increased considerably by the second date. On October 31, 1921, practically all of the larvæ of this species had left the ears, and those that remained were nearly all dead as a result of heavy frosts. The larvæ of the European corn borer, on the other hand, had continued feeding during this time, so that the damage that might be credited to this species was, by the last date, probably as great or greater than that done by the corn earworm. An actual count, like that of Table II, was not practicable, however, since it was not then possible to recognize the injury occasioned by each species.

Tables I and II show in what parts of the ears the larvæ of the two species were found and the damage that they had occasioned. For this purpose the ear has been divided into husk, peduncle, silk, cob, and grain. For the purpose of recording more accurately the exact region of injury to the grain with relation to the ear, the latter has been further divided into tip, middle, and butt; the "tip" being the upper third, the "middle" the middle third, and the "butt" the lower third of the ear.

TABLE I.—Infestation of field corn by the corn earworm, *Heliothis obsoleta* Fab., and the European corn borer, *Pyrausta nubilalis* Hbn., at Arlington, Mass., October 14, 1921

[100 ears of Longfellow flint field corn]

Ear No.	Number and location of larvæ found.											Part of ear infested or injured.					Total grains on ear.	Grains destroyed.
	Corn earworm.					European corn borer.												
	Husk.	Silk.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.		
1.			1			1	1						++	++	++	0	336	20
2.			2			1							++	++	++	0	272	0
3.			2			1							++	++	++	0	264	29
4.			2			1							++	++	++	0	256	0
5.			2	2		1			1				++	++	++	++	320	39
6.			1				1		1	1			++	++	++	++	240	3
7.		1	1										++	++	++	++	320	14
8.			2			2							++	++	++	++	256	30
9.				2									++	++	++	++	264	19
10.			1			1			1				++	++	++	++	416	35
11.			2					1					++	++	++	++	296	0
12.			2			3							++	++	++	++	240	28
13.			2	1		3				1	3		++	++	++	++	288	66
14.			1							1			++	++	++	++	384	12
15.									1	1			++	++	++	++	240	0
16.									1		2		++	++	++	++	256	32
17.			5			1			1				++	++	++	++	284	37
18.				2		1							++	++	++	++	208	47
19.			2			1				1			++	++	++	++	272	35
20.		2				1	1						++	++	++	++	336	0
21.						1			1	1			++	++	++	++	224	22
22.			3				1				2		++	++	++	++	224	48
23.			2							1			++	++	++	++	344	18
24.			2	1		1					4		++	++	++	++	264	36
25.			1										++	++	++	++	272	10
26.			2			1							++	++	++	++	344	10
27.				1									++	++	++	++	248	10
28.			3			4			1		1		++	++	++	++	240	50
29.		1	1										++	++	++	++	224	35
30.		3	2	2	1	1							++	++	++	++	224	53
31.	1					1			1				++	++	++	++	192	24
32.			2			1			1				++	++	++	++	208	2
33.			2										++	++	++	++	332	16
34.						1	1		1				++	++	++	++	320	0
35.			1			2							++	++	++	++	284	18
36.	1					1	3		1	3	2		++	++	++	++	344	22
37.			1			1	1				1		++	++	++	++	208	34
38.				1								1	++	++	++	++	368	20
39.			2		1		2					1	++	++	++	++	280	67
40.			2			2			1		1	1	++	++	++	++	216	24
41.										1			++	++	++	++	352	15
42.			1									2	++	++	++	++	232	24
43.						1			1	1			++	++	++	++	284	5



TABLE I.—*Infestation of field corn by the corn earworm, Heliothis obsoleta* Fab., and the European corn borer, *Pyrausta nubilalis* Hbn., at Arlington, Mass., October 14, 1921—Continued

Ear No.	Number and location of larvæ found.											Part of ear infested or injured.					Total grains on ear.	Grains destroyed
	Corn earworm.					European corn borer.												
	Husk.	Silk.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.	Tip.	Middle.	Butt.	Peduncle.	Husk.	Silk.	Cob.		
44.			2			1	1		1		2		+	+	+	+	284	10
45.						1	1		1	1	1		+	+	+	+	256	22
46.		1	1										+	+	+	+	192	51
47.									1		1		+	+	+	+	336	23
48.									1	3	1		+	+	+	+	320	25
49.			1			1	1	1	1		1		+	+	+	+	288	16
50.		2				1	1		2		1		+	+	+	+	320	15
51.						1		1	1				+	+	+	+	332	0
52.		1	1			1	1		1		1		+	+	+	+	284	74
53.						1		1	2	1			+	+	+	+	280	20
54.			2			1		1	1	1			+	+	+	+	280	36
55.		1	1			3			1	1			+	+	+	+	240	39
56.	1	1	1			1							+	+	+	+	368	29
57.	1	1	1										+	+	+	+	336	16
58.			2				1		1	1			+	+	+	+	256	24
59.			2						1		1		+	+	+	+	280	18
60.		1	2						1				+	+	+	+	360	15
61.			2										+	+	+	+	376	30
62.			2								1		+	+	+	+	288	56
63.		2										1	+	+	+	+	320	0
64.			1			2			2	1			+	+	+	+	256	40
65.			2			1			1				+	+	+	+	320	18
66.			2										+	+	+	+	392	32
67.			3			1							+	+	+	+	320	30
68.			1			1		1					+	+	+	+	256	8
69.			1			1			1				+	+	+	+	304	11
70.			1			1					1		+	+	+	+	240	23
71.				1					1			1	+	+	+	+	336	54
72.	1	1				1			1				+	+	+	+	384	35
73.		1				1	1		1	1	1		+	+	+	+	284	54
74.			3			1			1		1		+	+	+	+	336	92
75.		1	1			2			1				+	+	+	+	256	2
76.						1			1	1	2		+	+	+	+	328	44
77.			1			2	1						+	+	+	+	240	37
78.		2	1					1					+	+	+	+	208	26
79.			1				1		2	1	3		+	+	+	+	120	62
80.			2			1	1			1			+	+	+	+	256	43
81.		3	1			1	1		1	1	1		+	+	+	+	272	35
82.			2			2	1						+	+	+	+	288	36
83.			1			2	1		2		2		+	+	+	+	208	48
84.		1	1			1			1				+	+	+	+	344	15
85.		1	2			1	1		1				+	+	+	+	320	0
86.			2							1			+	+	+	+	262	51
87.			1	1		1			1		1		+	+	+	+	172	15
88.										1	1		+	+	+	+	172	47
89.		1	2						1				+	+	+	+	280	0
90.			1										+	+	+	+	256	12
91.											1		+	+	+	+	272	10
92.			1					4	1	2			+	+	+	+	272	47
93.				1		1			2		1	1	+	+	+	+	286	37
94.			2										+	+	+	+	272	51
95.			1						1		2		+	+	+	+	320	102
96.			3	1					1		1		+	+	+	+	288	44
97.			2			1	1						+	+	+	+	280	38
98.		1	1	1					1		2		+	+	+	+	286	54
99.		1	1								1		+	+	+	+	272	48
100.			1			1					1		+	+	+	+	272	48

## Per cent of ears infested:

By either species.....	per cent.	100
By both species.....	do.	71
By European corn borer.....	do.	88
By corn earworm.....	do.	83

## Average number of corn earworms per infested ear.....

Average number of European corn borers per infested ear..... 2.92

Total grain destroyed by both species..... per cent. 10.5

Per cent of stalks infested (23 stalks)..... do. 100

## Infestation per stalk:

Greatest..... larvæ.. 21

Least..... do. 4

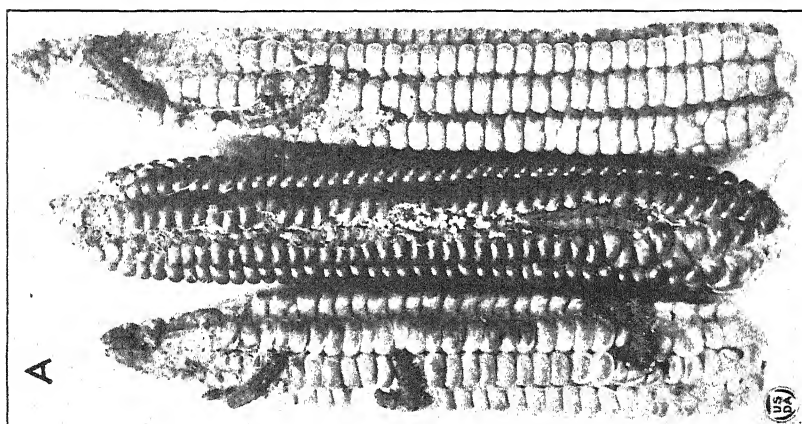
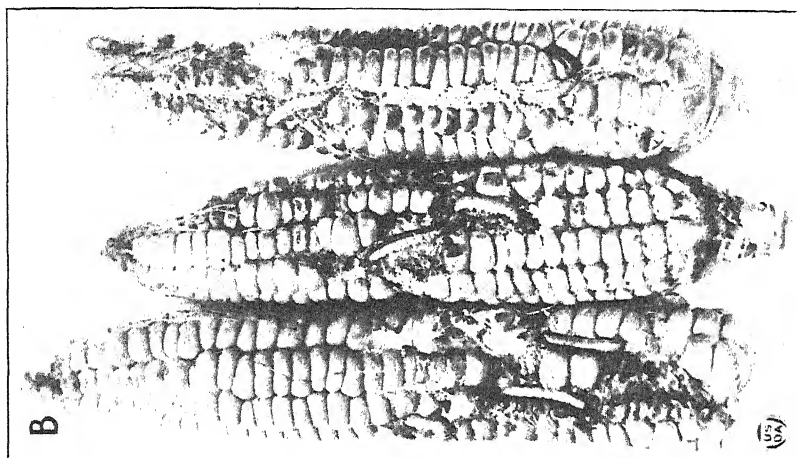
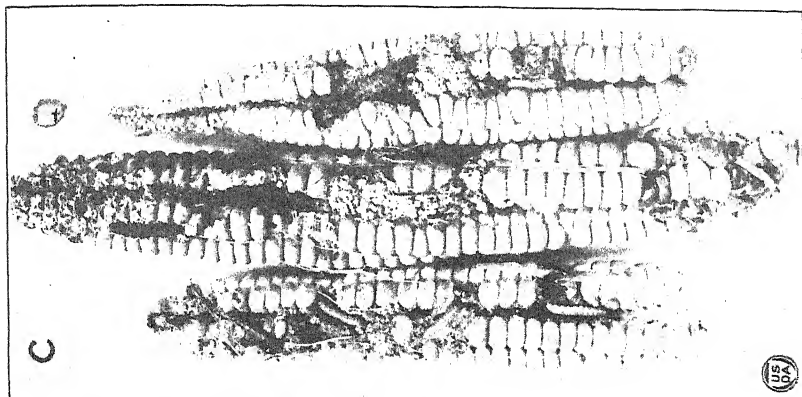
Average..... do. 9.82

Greatest grain injury on any one ear..... per cent. 31.9

PLATE I

*Pyrausta nubilalis* and *Heliothis obsoleta*

- A.—Ears of Longfellow flint field corn injured by larvæ of *Heliothis obsoleta*.
- B.—Ears of Longfellow flint field corn injured by larvæ of *Pyrausta nubilalis*.
- C.—Ears of Longfellow flint field corn injured by larvæ of *Heliothis obsoleta* and of *Pyrausta nubilalis*.





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No. 2

## ANCHORAGE AND EXTENT OF CORN ROOT SYSTEMS<sup>1</sup>

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### INTRODUCTION

Results from preliminary experiments indicate that there are fundamental differences in the root systems of various inbred strains of corn. The object of the present paper is (1) to call attention to a plant-pulling machine which was designed for these studies and (2) to point out a few significant differences in the root systems of the inbred strains that were included in the experiments.

### MATERIALS AND METHODS

A plant-pulling machine (Pl. 1) was designed to measure the resistance of individual plants or hills of corn to a vertical pull. The essential features of this machine are a cross beam with three axes which divide the beam into two parts, so as to make a lever of the second class. Each axis consists of a knife edge turning in a clevis. The distal axis is attached to a spring balance which registers the pulling resistance to the tenth of a pound. The clevis of the other end of the beam is supported by a block and tackle, the rope being pulled by winding it on a 2-inch drum. Two thicknesses of strong cotton webbing such as are used for trunk straps were attached to the third clevis, and these were attached to the base of the corn stalks by a suitable hitch.

The inbred strains of corn, except where indicated otherwise in the tables, had been selfed five and six years. The strain, designated as "good," not only possessed the ability to stand erect under adverse weather conditions, but was exceptionally good in general vigor, chlorophyll development, and resistance to fungus diseases, including root-rot and smut. The strain susceptible to rootrot was equally good in general vigor, and the leaves were free from leaf spotting and other chlorophyll deficiencies, but plants of this strain lodged badly during windstorms. At no time did the leaves of either of the above strains show any tendency to wilt during early or mid-season periods. Leaves of the strain susceptible to leaf firing invariably wilted during any dry, hot period in July. Severe wilting of the leaves was followed by a dying of portions of those leaves.

The field data were secured from plats having a perfect stand of one plant every 22 inches in rows 42 inches apart. The plants grown in the greenhouse were spaced 18 inches each way.

The significance of differences in means was determined by "Student's" method,<sup>3</sup> wherever that method could be applied. Differences with odds of 30:1, were considered significant.

<sup>1</sup> Accepted for publication Nov. 19, 1923.

<sup>2</sup> The investigations reported in this paper were conducted in cooperation with the Illinois Agricultural Experiment Station and Funk Bros. Seed Co., of Bloomington, Ill.

<sup>3</sup> THE PROBABLE ERROR OF A MEAN. *In* Biometrika, v. 6, p. 1-25. 1908. (Signed by Student.)

## EXPERIMENTAL DATA

## ROOT ANCHORAGE STUDIES

The results presented in Table I were secured under very uniform conditions. Previous to planting the corn, the large soil pit in one of the greenhouses at the University of Illinois was refilled with new soil which had been thoroughly mixed. Although plants of the strain susceptible to rootrot had not been exposed to wind and storm and had equal opportunities to develop a strong root system, yet the mean pull of these plants was less than half that of plants of the good strain,  $13.8 \pm 1.1$  pounds compared with  $29.8 \pm 1.8$  pounds, respectively. Plants of the strain susceptible to leaf firing offered less than one-third the resistance to a vertical pull that was exhibited by plants of the good strain,  $9.1 \pm 0.9$  pounds compared with  $29.8 \pm 1.8$  pounds, respectively. There was no material difference in the height of plants of these three strains on the date when the plants were pulled. None of the roots showed any evidence of root rotting. Leaves of the strain susceptible to leaf firing apparently were normal in every respect. In view of such facts, the differences of  $16.0 \pm 2.1$  pounds and  $20.7 \pm 2.0$  pounds in root anchorage are very significant. The two first generation hybrids were somewhat more vigorous than the good inbred strain as measured by vegetative growth, but they were not superior in pulling resistance. These data suggest that the genetic factors responsible for the reduced root systems in the strains susceptible to rootrot and leaf firing, respectively, are recessive. Repeated field experiments not herein reported confirm this suggestion.

The plants of a number of inbred strains were pulled after they had matured in field plots, but while the stalks were still green. The results given in Table II show that erect plants of the good strain were better anchored than erect plants of the strain susceptible to rootrot. The difference of 48.6 pounds, or 23.4 per cent, with odds of 132:1, is sufficiently large to be significant.

TABLE I.—Data on the root anchorage of three unrelated selfed strains of Yellow Dent and three  $F_1$  crosses measured by their resistance to a vertical pull, the corn being planted Mar. 8, 1922, in the greenhouses of the University of Illinois and pulled May 6, 1922

Character of inbred strains and crosses.	Number of plants pulled.	Mean pulling resistance per plant.	Difference in pulling resistance based on good strain.		Difference P. E.
			Pounds.	Per cent.	
Good.....	23	$29.8 \pm 1.8$			
Susceptible to rootrot.....	22	$13.8 \pm 1.1$	$16.0 \pm 2.1$	53.7	7.6
Susceptible to leaf firing.....	25	$9.1 \pm 0.9$	$20.7 \pm 2.0$	69.5	10.3
$F_1$ (Good x rootrot susceptible).	26	$29.4 \pm 1.6$	$.4 \pm 2.4$	1.3	.2
$F_1$ (Good x leaf firing susceptible)	26	$28.3 \pm 1.6$	$1.5 \pm 2.4$	5.0	.7

TABLE II.—Data on root anchorage of twenty-eight erect plants of a good inbred strain and a similar number of erect plants in an adjacent row of an inbred strain susceptible to rootrot, the corn being planted May 30, 1922, near Bloomington, Ill., in brown silt loam soil, and root anchorage data taken September 26, 1922

Character of strain.	Number of plants pulled.	Mean pulling resistance per plant.	Difference in pulling resistance.		Odds.
		Pounds.	Pounds.	Per cent.	
Good.....	28	208.0			
Susceptible to rootrot.....	28	159.4	48.6	23.4	132:1

The relation of root anchorage to lodging is shown by data presented in Table III. As the mean pulling resistance of erect plants decreased,

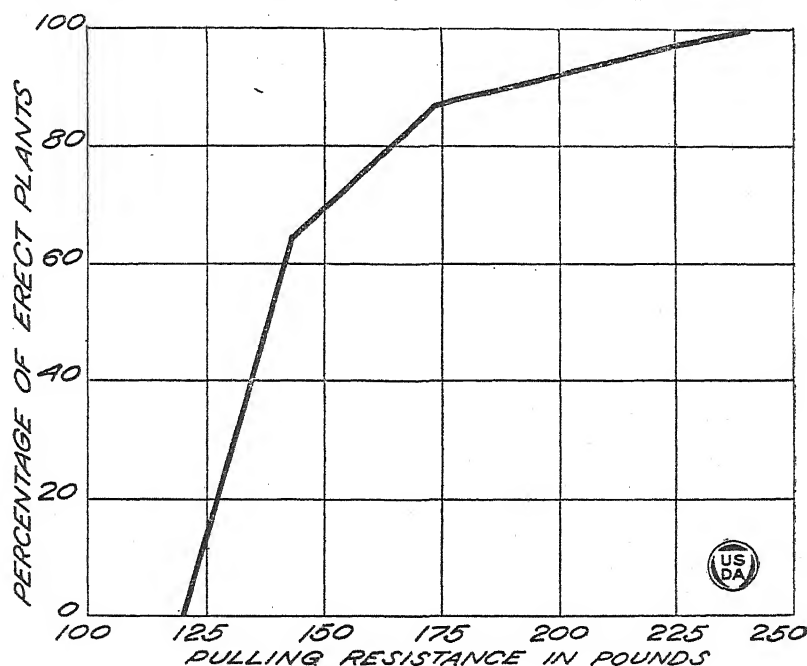


FIG. 1.—Graphic representation of data in Table III, showing increase in force necessary to uproot corn plants as the percentage of erect plants increased.

the percentage of leaning plants increased. These data are presented graphically in figure 1.

Strains which have the ability to stand erect throughout the season may vary greatly in their resistance to a vertical pull, as shown by data presented in Table IV. The difference of 32.7 pounds, or 9 per cent, in pulling resistance between G-4-2 and G-4-3 is not significant in view of the small odds involved. The difference of 118.9 pounds, or 32.9 per cent, however, between G-4-2 and G-4-4, with odds of 999:1, is very significant.

TABLE III.—Data on relation of anchorage to lodging in some closely related inbred strains of Yellow Dent corn, as measured by their resistance to a vertical pull, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and plants pulled September 27-28

Pedigree number.	Number of plants pulled.	Mean plant height.	Plants leaning 30° or more.		Mean pulling resistance per plant.		Difference in mean pulling resistance per plant based on B-1-1-1-R-3.			
					Erect plants.	Mean of both erect and leaning plants.	Erect plants.	Difference/ P. E.	Both erect and leaning plants.	Difference/ P. E.
		Inches.	Number.	Per cent.	Pounds.	Pounds.	Pounds.		Pounds.	
B-1-1-1-R-3...	37	82.6±1.1	0	0.0	240.0±7.1	240.0±7.1				
B-1-1-1-R-7...	49	96.0±0.8	1	2.5	328.0±6.1	224.6±5.0	12.0±9.4	1.3	15.4±9.3	1.6
B-1-1-1-R-8...	39	95.4±1.3	5	13.9	183.9±9.4	173.0±9.0	56.1±11.8	4.7	67.0±11.5	5.8
B-1-1-1-R-10...	39	87.3±0.7	14	35.9	155.3±10.0	144.3±7.5	84.7±12.2	6.9	95.7±10.3	9.3
B-1-1-1-2...	25	89.6±1.0	25	100.0		120.1±8.1			119.9±10.8	11.1

TABLE IV.—Data on root anchorage of 25 erect plants from each of three closely related inbred strains of Yellow Dent corn grown in contiguous rows, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and root anchorage data taken September 28

Pedigree number.	Number of plants.	Mean pulling resistance per plant.	Difference in mean pulling resistance per plant based on G-4-2.		Odds.
		Pounds.	Pounds.	Per cent.	
G-4-2.....	25	361.5			
G-4-3.....	25	328.8	32.7	9.0	5 : 1
G-4-4.....	25	242.6	118.9	32.9	999 : 1

### EXTENT OF ROOT SYSTEMS

Root counts on plants grown under field conditions are given in Table V. Plants of the strain susceptible to rootrot and also of the strain susceptible to leaf firing had a significantly smaller number of main roots than plants of the good strain,  $20.0 \pm 1.6$  roots and  $15.4 \pm 0.7$  roots compared with  $33.0 \pm 0.8$  roots, respectively (Pl. 2). There was no real difference in mean plant height of the three strains. Such data not only throw considerable light on the root-anchorage data presented in Table I, but suggest a fundamental cause for the initial wilting of the leaves of the strain susceptible to leaf firing. In this strain there seemed to be an actual deficiency in the root system compared to the vegetative growth above ground. During periods of rather low soil moisture and high transpiration, such a small root system could hardly be expected to supply the needs of the plant. Differences in the root systems of the good strain and the strain susceptible to leaf firing are illustrated in Plate 2.

The results from root examinations made August 17-25 are given in Table VI. There was a great increase in growth in all parts of the plant during the period between July 18 and August 17-25. The great increase in number of roots is in accord with the findings of Weaver, Jean, and Crist.<sup>4</sup>

<sup>4</sup> WEAVER, John E., JEAN, Frank C., and CRIST, John W. DEVELOPMENT AND ACTIVITIES OF ROOTS OF CROP PLANTS. VI, 117 p., 42 fig., 14 pl. Washington, D. C. 1922. Bibliography, p. 116-117. (Carnegie Inst. Wash. Pub. 316.)



TABLE V.—Data from a comparative study of the root systems of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated July 18, 1922

Character of inbred strains.	Number of plants examined.	Mean plant height.	Mean number main roots per plant.	Difference in number of main roots per plant based on good strain.		Difference/P.E.
				Number.	Per cent.	
Good.....	4	Inches. 59.0±1.1	33.0±0.8	.....	.....	.....
Susceptible to rootrot..	7	59.3±1.0	20.0±1.6	13.0±1.8	39.4	7.2
Susceptible to leaf firing.....	9	57.2±2.4	15.4±0.7	17.6±1.1	53.3	16.0

TABLE VI.—Data from a comparative study of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated August 17-25

Character of strain.	Number of plants examined.	Mean height.	Mean circumference.	Mean number of main roots.	Mean percentage of rotted roots 10 inches below surface.	Mean number of lateral roots per plant on six 6-inch pieces of main roots.		Mean total length of all lateral roots per plant on six 6-inch pieces of main roots.	
						2-8 inches below surface.	10-16 inches below surface.	2-8 inches below surface.	10-16 inches below surface.
Good.....	9	Inches. 99.0	Inches. 3.41	58.5	9.7	391.0	244.6	Inches. 774.7	Inches. 423.6
Susceptible to rootrot..	9	109.1	3.45	51.7	96.7	293.1	96.0	334.4	103.5
Susceptible to leaf firing.....	9	102.0	3.40	57.0	4.0	391.0	160.2	423.7	208.9
Difference between good strain and strain susceptible to rootrot .....		10.1	.....	6.8	87.0	97.9	168.6	330.3	320.1
Percentage difference between good strain and strain susceptible to rootrot .....		10.2	.....	11.6	.....	25.0	69.0	43.1	75.6
Odds.....	9,999:1			59:1	525:1	1,492:1	348:1	1,999:1	4,544:1
Difference between good strain and strain susceptible to leaf firing.....							84.4	351.0	214.7
Percentage difference between good strain and strain susceptible to leaf firing....							34.5	45.3	50.6
Odds.....							344:1	81:1	1,350:1

The difference of 6.8 in mean number of main roots between the good strain and the strain susceptible to rootrot, although not large, is rather significant in view of the odds of 59 to 1. Plants of the strain susceptible to leaf firing showed a very large gain in number of main roots during the period between July 18 and August 25. The number of rotted roots in the strain susceptible to rootrot was very pronounced. There was little evidence of rootrot in the strain susceptible to leaf firing.

TABLE III.—Data on relation of anchorage to lodging in some closely related inbred strains of Yellow Dent corn, as measured by their resistance to a vertical pull, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and plants pulled September 27-28

Pedigree number.	Number of plants pulled.	Mean plant height.	Plants leaning 30° or more.	Mean pulling resistance per plant.		Difference in mean pulling resistance per plant based on B-1-1-1-R-3.			
				Erect plants.	Mean of both erect and leaning plants.	Erect plants.	Difference/ P. E.	Both erect and leaning plants.	Difference/ P. E.
		Inches.	Number.	Per cent.	Pounds.	Pounds.	Pounds.	Pounds.	
B-1-1-1-R-3..	37	82.6±1.1	0	0.0	240.0±7.1	240.0±7.1	.....	.....	.....
B-1-1-1-R-7..	40	96.0±0.8	1	2.5	228.0±6.1	224.6±6.0	12.0±9.4	1.3	15.4±9.3
B-1-1-1-R-8..	36	95.4±1.3	5	13.9	183.9±9.4	173.0±9.0	56.1±11.8	4.7	67.0±11.5
B-1-1-1-R-10.	39	87.3±0.7	14	35.9	253.3±10.0	144.3±7.5	84.7±12.2	6.9	95.7±10.3
B-1-1-1-R-12..	28	89.6±1.0	25	100.0	.....	120.1±8.1	.....	.....	119.9±10.8
									11.1

TABLE IV.—Data on root anchorage of 25 erect plants from each of three closely related inbred strains of Yellow Dent corn grown in contiguous rows, the corn being planted May 22, 1922, near Bloomington, Ill., in brown silt loam soil, and root anchorage data taken September 28

Pedigree number.	Number of plants.	Mean pulling resistance per plant.	Difference in mean pulling resistance per plant based on G-4-2.		Odds.
			Pounds.	Per cent.	
G-4-2.....	25	361.5	.....	.....	.....
G-4-3.....	25	328.8	32.7	9.0	5 : 1
G-4-4.....	25	242.6	118.9	32.9	999 : 1

#### EXTENT OF ROOT SYSTEMS

Root counts on plants grown under field conditions are given in Table V. Plants of the strain susceptible to rootrot and also of the strain susceptible to leaf firing had a significantly smaller number of main roots than plants of the good strain,  $20.0 \pm 1.6$  roots and  $15.4 \pm 0.7$  roots compared with  $33.0 \pm 0.8$  roots, respectively (Pl. 2). There was no real difference in mean plant height of the three strains. Such data not only throw considerable light on the root-anchorage data presented in Table I, but suggest a fundamental cause for the initial wilting of the leaves of the strain susceptible to leaf firing. In this strain there seemed to be an actual deficiency in the root system compared to the vegetative growth above ground. During periods of rather low soil moisture and high transpiration, such a small root system could hardly be expected to supply the needs of the plant. Differences in the root systems of the good strain and the strain susceptible to leaf firing are illustrated in Plate 2.

The results from root examinations made August 17-25 are given in Table VI. There was a great increase in growth in all parts of the plant during the period between July 18 and August 17-25. The great increase in number of roots is in accord with the findings of Weaver, Jean, and Crist.<sup>4</sup>

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TABLE V.—Data from a comparative study of the root systems of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated July 18, 1922

Character of inbred strains.	Number of plants examined.	Mean plant height.	Mean number main roots per plant.	Difference in number of main roots per plant based on good strain.		Difference/P.E.
				Number.	Per cent.	
Good.....	4	Inches. 59.0±1.1	33.0±0.8	.....	.....	.....
Susceptible to rootrot..	7	59.3±1.0	20.0±1.6	13.0±1.8	39.4	7.2
Susceptible to leaf firing.....	9	57.2±2.4	15.4±0.7	17.6±1.1	53.3	16.0

TABLE VI.—Data from a comparative study of three unrelated inbred strains of Yellow Dent corn planted June 2, 1922, at Bloomington, Ill., in brown silt loam soil that previously had produced two crops of corn, and excavated August 17-25

Character of strain.	Number of plants examined.	Mean height.	Mean circumference.	Mean number of main roots.	Mean percentage of rotted roots 10 inches below surface.	Mean number of lateral roots per plant on six 6-inch pieces of main roots.		Mean total length of all lateral roots per plant on six 6-inch pieces of main roots.	
						2-8 inches below surface.	10-16 inches below surface.	2-8 inches below surface.	10-16 inches below surface.
Good.....	9	Inches. 99.0	Inches. 3.41	58.5	9.7	391.0	244.6	774.7	423.6
Susceptible to rootrot..	9	109.1	3.45	51.7	96.7	293.1	96.0	334.4	103.5
Susceptible to leaf firing.....	9	102.0	3.40	57.0	4.0	391.0	160.2	423.7	208.9
Difference between good strain and strain susceptible to rootrot .....	.....	10.1	.....	6.8	87.0	97.9	168.6	330.3	320.1
Percentage difference between good strain and strain susceptible to rootrot .....	.....	10.2	.....	11.6	.....	25.0	69.0	43.1	75.6
Odds.....	.....	9,999:1	.....	59:1	525:1	11,492:1	348:1	1,999:1	4,544:1
Difference between good strain and strain susceptible to leaf firing.....	.....	.....	.....	.....	.....	.....	84.4	351.0	214.7
Percentage difference between good strain and strain susceptible to leaf firing. ....	.....	.....	.....	.....	.....	.....	34.5	45.3	50.6
Odds.....	.....	.....	.....	.....	.....	.....	344:1	81:1	1,350:1

The difference of 6.8 in mean number of main roots between the good strain and the strain susceptible to rootrot, although not large, is rather significant in view of the odds of 59 to 1. Plants of the strain susceptible to leaf firing showed a very large gain in number of main roots during the period between July 18 and August 25. The number of rotted roots in the strain susceptible to rootrot was very pronounced. There was little evidence of rootrot in the strain susceptible to leaf firing.

Actual differences in root systems of the three strains could not be expressed fully by the number of main roots; there were significant differences both in number and length of lateral roots (Plate 3). There was a very significant reduction in number of lateral roots in the strain susceptible to rootrot, both 2 to 8 inches below the surface and 10 to 16 inches below the surface. The figures were 293.1 and 96 lateral roots compared with 391 and 244.6 roots, respectively. (Table VI.) Plants of the strain susceptible to leaf firing developed as many lateral roots near the surface as plants of the good strain, but there was a marked reduction in the number of lateral roots 10 to 16 inches below surface, or 160.2 lateral roots compared with 244.6 roots. Differences in total length of the lateral roots on the same number of main roots of an equal length were even more marked than differences in the number of lateral roots, and perhaps were more important from the standpoint of the physiology of the plants concerned. In the strain susceptible to rootrot there was less than half the total length of lateral roots 2 to 8 inches below surface and only one-fourth the total length 10 to 16 inches below the surface, or 334.4 inches and 103.5 inches compared with 774.7 inches and 423.6 inches, respectively. (Table VI.) Roots of the strain susceptible to leaf firing were decidedly inferior to roots of the good strain in this respect, or 423.7 inches and 208.9 inches compared with 774.7 inches and 423.6 inches, respectively. All the differences in mean total length of all laterals on equal lengths of main roots from the different strains were significant from the standpoint of statistical analysis.

TABLE VII.—Data from a comparative study of 10 plants of a good inbred strain of Yellow Dent corn and 10 and 5 plants, respectively, from two unrelated inbred strains susceptible to rootrot, planted May 22, near Bloomington, Ill., in brown silt loam soil that previously had produced three consecutive crops of corn, and excavated September 11-14, 1922

Character of strain.	Number of plants.	Mean plant height.	Mean plant circumference at base.	Mean total number of main roots per plant.	Mean total number of lateral roots per plant on six 6-inch pieces of main roots 6-12 inches below surface.	Mean total length of all lateral roots per plant on six 6-inch pieces of main roots 6-12 inches below surface.	Mean total length of all laterals per plant on healthy roots only, in a horizontal 6-inch section 6-12 inches below surface.
		Inches.	Inches.			Inches.	Inches.
Good.....	10	82.8 ± 1.2	3.78 ± 0.13	41.2 ± 0.4	434.5 ± 33.5	706.1 ± 91.9	4,343.9 ± 415.1
(a) Susceptible to rootrot.....	10	102.3 ± 1.2	3.69 ± 0.11	35.1 ± 2.0	297.6 ± 16.1	258.8 ± 21.8	1,220.2 ± 130.7
(b) Susceptible to rootrot.....	5	90.8 ± 3.7	3.70 ± 0.12	31.0 ± 1.1	209.8 ± 23.0	208.6 ± 35.1	946.8 ± 153.8
Difference between good and (a) susceptible strain.....		19.5 ± 1.7	0.09 ± 0.2	6.1 ± 2.0	136.9 ± 37.2	447.3 ± 94.3	3,123.7 ± 435.2
Percentage difference between good strain and (a) susceptible strain.....		23.5		14.8	31.5	63.3	72.2
Difference/P. E.....		11.5		3.0	3.7	4.7	7.2
Difference between good and (b) susceptible strain.....		8.0 ± 3.9	0.08 ± 0.2	10.2 ± 1.2	224.7 ± 40.6	497.5 ± 98.4	3,397.1 ± 482.5
Percentage difference between good strain and (b) susceptible strain.....							
Difference/P. E.....		9.7		24.8	51.7	70.4	78.2
		2.1		8.5	5.5	5.1	7.0

Additional data from a comparative study of root systems of plants from a good strain and two strains susceptible to rootrot are given in Table VII. Differences in the number of main roots,  $6.1 \pm 2.0$  roots and  $10.2 \pm 1.2$  roots, were marked. The latter difference, being 8.5 times its probable error, is very significant. Both differences in mean total number and mean total length of all laterals on equal lengths of main roots are very significant. The greatest and most important difference between the good strain and the strains susceptible to rootrot, however, lay in the mean total lengths of all laterals per plant on healthy roots only in a horizontal 6-inch section 6 to 12 inches below the surface. In one comparison the difference was 7.2 times the probable error, and in the other comparison, 7.0 times the probable error.

The significance of such differences in root systems is still further emphasized by the fact that plants of the strains susceptible to rootrot were considerably taller than plants of the good strain. The importance of such differences in ratio of lengths of roots which function to those of parts above ground is plainly evident.

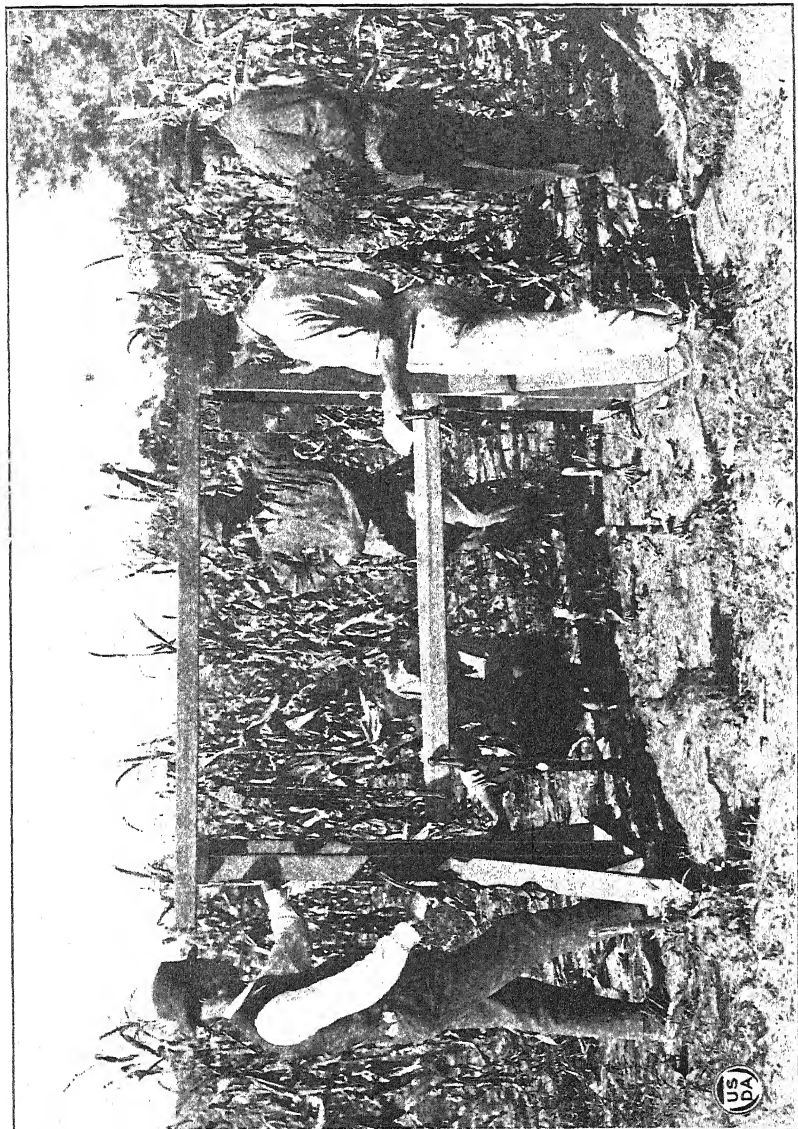
Additional data on the relation of extent of root system to pulling resistance and lodging in the good strain and the strain susceptible to rootrot are presented in Table VIII. The difference of 6, or 16.2 per cent, in number of main roots, although not large, was very consistent and significant. Differences both in mean total number of lateral roots and mean total length of lateral roots were very marked. The strain susceptible to rootrot had a lower pulling resistance and more leaning plants than the good strain.

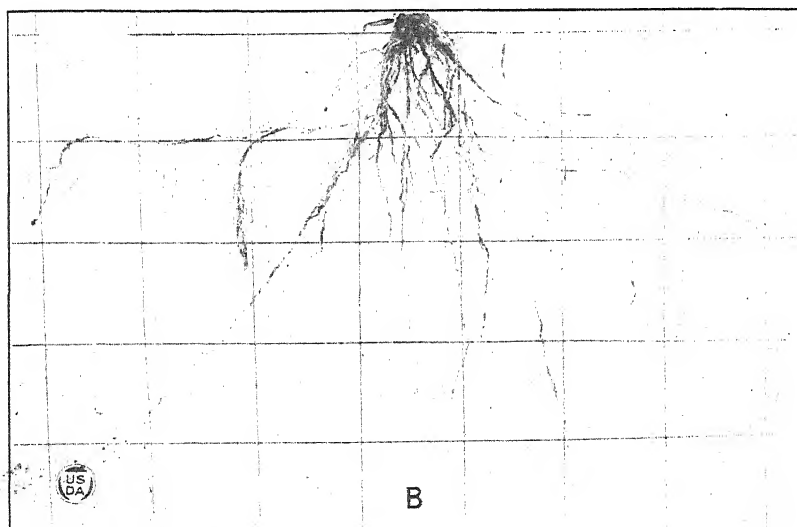
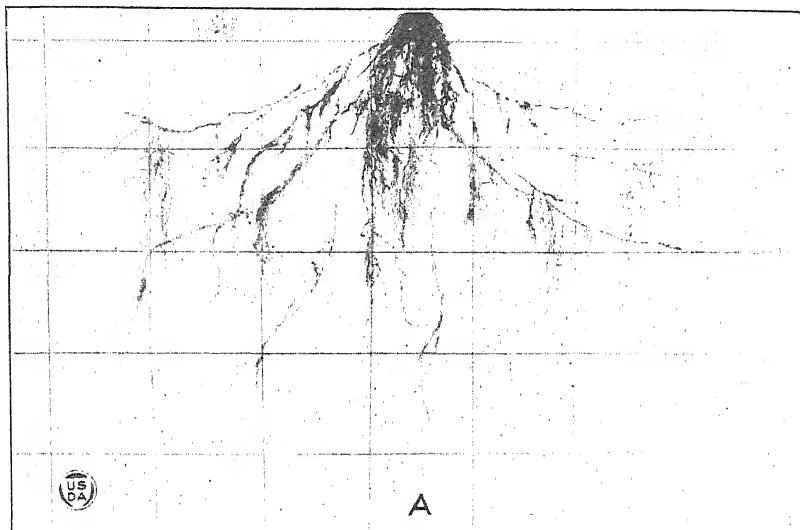
TABLE VIII.—Data from a comparative study of plants from a good inbred strain and plants from an unrelated inbred strain susceptible to rootrot, the corn being planted May 22, 1922, in adjacent rows, near Bloomington, Ill., on brown silt loam soil on which the three previous crops were corn, 10 plants from each strain being excavated September 16-18, and 17 erect plants with green stalks from each strain being pulled September 28 and 29

Character of strain.	Percentage of plants leaning 30° or more in a period of six years.						Mean resistance per plant to a vertical pull.	Mean number of main roots per plant.	Mean total number of lateral roots per plant on six 6-inch pieces of main roots 6 to 12 inches below surface.	Mean total length of lateral roots per plant on six 6-inch pieces of main roots 6 to 12 inches below surface.
	1917	1918	1919	1920	1921	1922				
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Pounds.			Inches.
Good.....	0.0	8.9	0.0	0.0	7.8	2.5	251.5	37	318.4	434.5
Susceptible to rootrot.....	0.0	7.1	31.8	10.1	83.3	20.0	198.5	31	212.7	217.4
Difference between good and susceptible strains.....							53.0	6	105.7	217.1
Percentage difference based on good strain.....							21.1	16.2	33.2	50.0
Odds.....							322:1	3,332:1	554:1	908:1

#### PLATE I

The root-pulling machine in operation. The corn stalks were first cut off at a convenient height. The upright standard next to the scale is open in the center at the bottom so that the corn stumps pass through it and the machine need not be lifted over them. The scale used was a Chatillon spring balance graduated in tenths of pounds with a capacity of 120 pounds at the scale. The maximum pulling resistance measured so far was 865 pounds. The scale is hooked to the beam by means of a chain so that it can be hooked at any height which will bring the beam into a horizontal position when the greatest strain is being exerted.







## PLATE 2

A.—Representative root system of the good strain of corn. The corn was planted June 2, 1922, and excavated when the plant was 46 days old. Previous to photographing, the roots were mounted on a frame with wires stretched at right angles 6 inches apart each way. On the frame the main roots were placed, as nearly as possible, in their original position in relation to a vertical line passing through the center of the base of the plant.

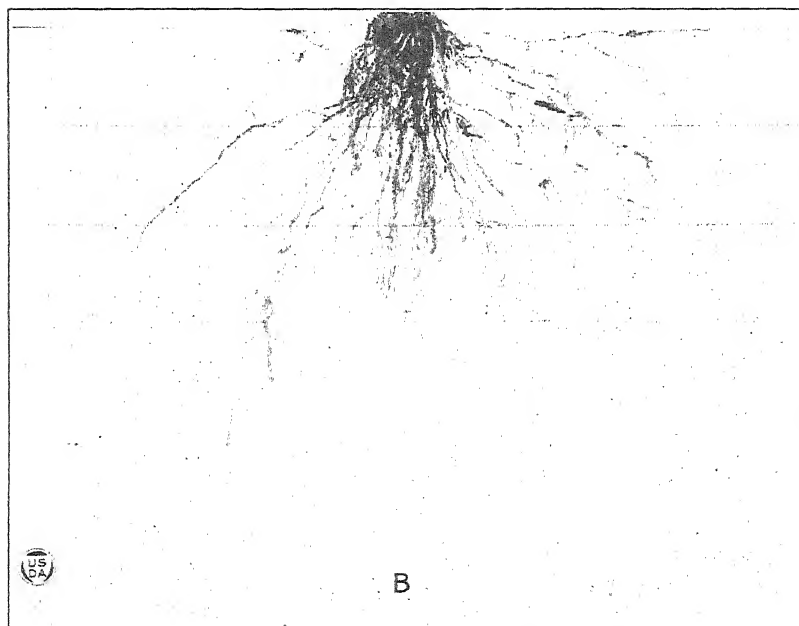
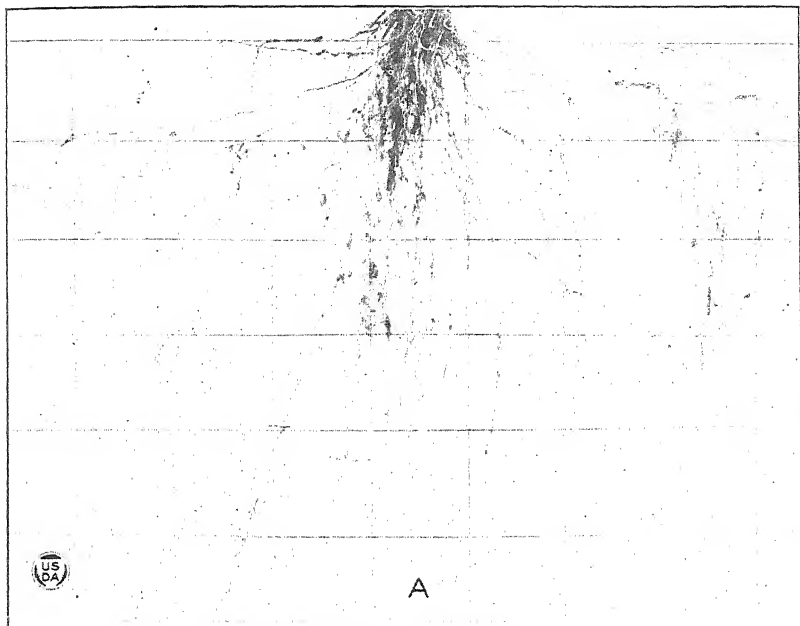
B.—Representative root system of a strain of corn susceptible to leaf firing. The corn was planted June 2 in a row adjacent to the good strain, and excavated when the plant was 46 days old. At the time the excavations were made the leaves of the strain susceptible to leaf firing were beginning to wilt, although there was no evidence of root rotting. Other similar plants of this same strain partially recovered after rains that broke the drought.

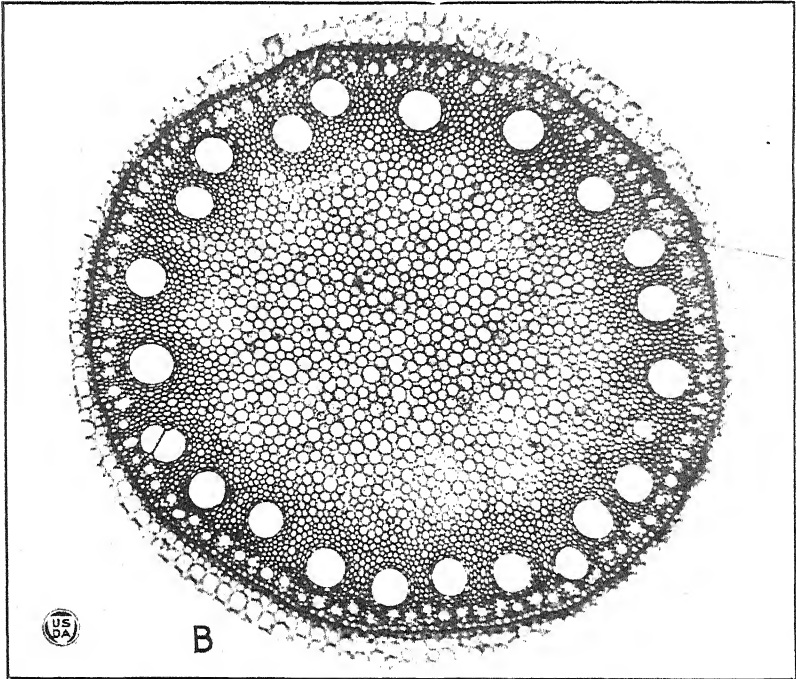
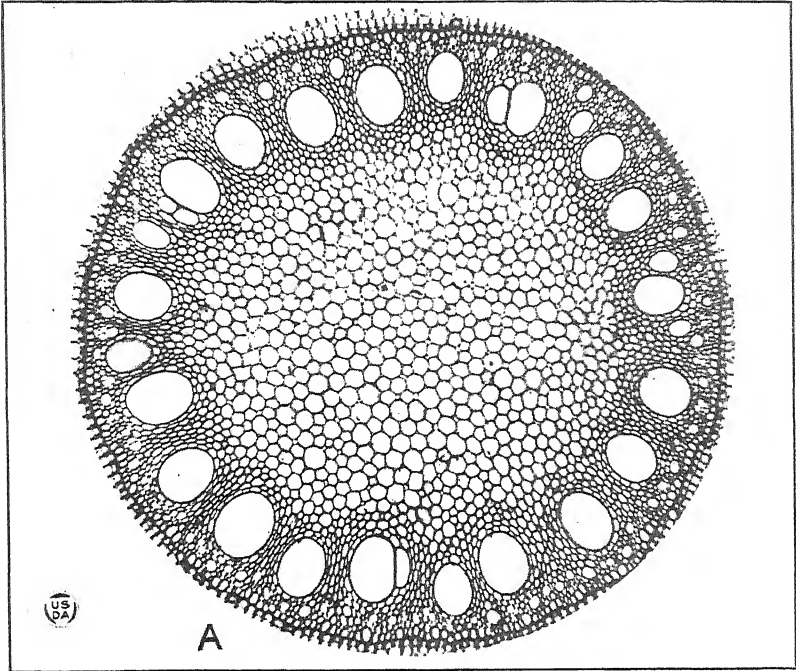
The differences in the two types of root systems are very marked, both in number of main roots and number and length of lateral branches.

### PLATE 3

A.—Representative root system of a good inbred strain of corn. The corn was planted June 2, 1922, and was excavated 77 days after planting. Less than 10 per cent of the roots showed evidence of root rotting.

B.—Representative root system of an inbred strain of corn susceptible to rootrot. Over 96 per cent of the roots showed evidence of root rotting.





#### PLATE 4

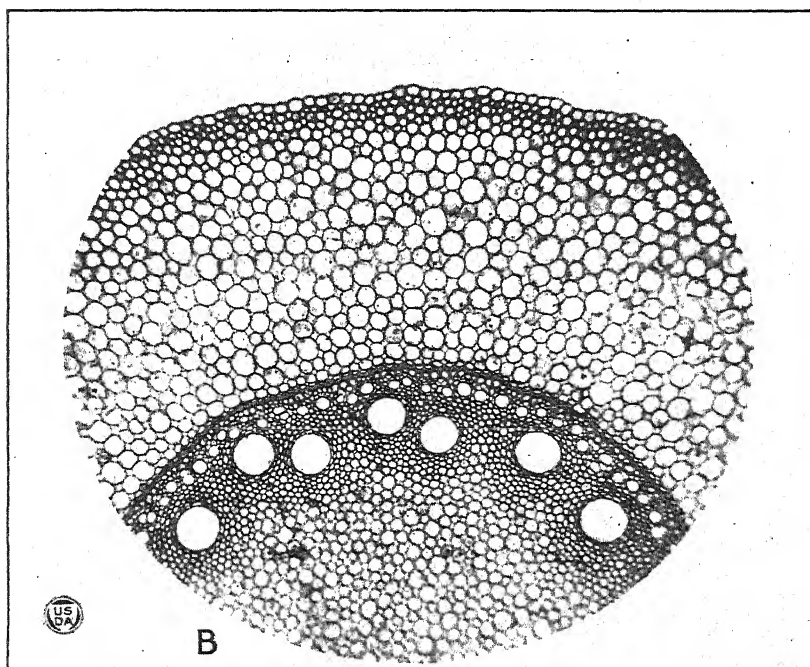
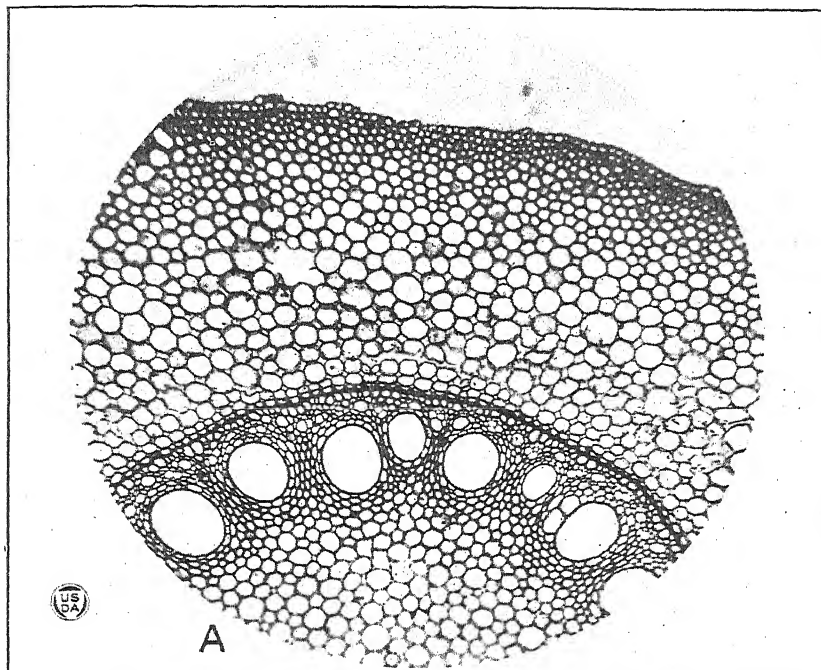
A.—Cross section of central stele of the root of a good inbred strain of Yellow Dent corn that was unusually vigorous, had no chlorophyll deficiencies, yielded well, and was highly resistant to rootrot and smut. The tracheids are large and the pith cells are closely bound together with thickenings at the corners.

B.—Cross section of central stele of the root of an inbred strain of Yellow Dent corn that possessed the other desirable qualities mentioned above but was susceptible to rootrot and lodged easily. The tracheids are comparatively small and the pith cells are very loosely connected so that they are round in cross section, whereas those above are angular. The spaces between the cells are open, and there are no special thickenings.

## PLATE 5

A.—Cross section of the cortex of a good inbred strain, the same as shown in Plate 4, A. The cells are thick walled and are closely bound together, which gives the cells an angular shape in cross section. Many of the corners are reinforced by special thickenings.

B.—Cross section of the cortex of an inbred strain which is susceptible to rootrot, the same as shown in Plate 4, B. The cells are not as thick walled as those above; they are round in cross section, and are not closely bound together.







# SOYBEAN MOSAIC: SEED TRANSMISSION AND EFFECT ON YIELD<sup>1</sup>

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## INTRODUCTION

Rather extensive inoculation tests in field and greenhouse have failed to reveal any host for soybean mosaic other than the soybean itself. Cross inoculations from mosaic red clover and garden bean to soybean were unsuccessful, and cross inoculations from soybean to sixty varieties of garden beans and seven other species of *Phaseolus*, to two species of *Dolichos*, to field peas, and to cowpeas gave only negative results.

Mosaic has been noted on the following varieties of soybeans at La Fayette, Ind.: Midwest or Medium Yellow, Haberlandt, Manchu, Ito San, Mongol, Hurreibrinks, Mammoth Black, Habara, A. K., Arlington, Hoosier, Elton, Wea, Lexington, Black Eyebrow, Pinpu, 36847, Feldun, Dunfield, Soysota, Wilson Black, Mammoth Yellow, Brown, Virginia, and Tar Heel Black. The disease seems to be most prevalent in the Midwest, Haberlandt, and Black Eyebrow varieties and the symptoms seem to be most conspicuous in the Midwest variety. The Midwest or Medium Yellow variety has been erroneously known locally as Hollybrook, according to Wiancko and Mulvey (9),<sup>3</sup> and it was in this variety that we (5) first noted the disease.

As yet the mosaic disease does not seem to have become seriously prevalent in Indiana. F. E. Robbins found the disease in only 4 out of 27 soybean fields inspected in 1923. With the exception of one field of the Midwest variety, only a very low percentage of infection has been found in commercial fields.

In addition to the typical mosaic symptoms described in a previous account by the authors (5), other symptoms have been found associated with the disease, but these have not been relied upon as criteria of mosaic in the following work. Among these symptoms are a bronzing of the young leaves produced by a brown discoloration of short segments of the veins and large splotches on the older leaves produced by a lacelike yellowing or browning of the veins. In the Lexington variety severe symptoms were evinced in 1923. The mosaic plants were extremely stunted, the growing tips were killed outright, and brown necrotic streaks developed on the stems and petioles.

## SEED TRANSMISSION

In the previous account (5) it was recorded that about 13 per cent of the seed from mosaic plants transmitted the disease. Subsequent tests have given similar results with a number of varieties and with older seed.

The presence of the disease in ordinary commercial seed one year old was shown in 1921. Fifteen varieties and selections were planted in

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 98.

parallel plots of three rows each on May 26, 1921, and the occurrence of a very small percentage of seed-borne mosaic in 9 of the 15 varieties was determined by a careful examination of the seedlings on June 26 as shown in Table I. The secondary spread of infection as shown by the records of August 16 will be referred to later.

TABLE I.—*Mosaic in variety plot, 1921*

Variety.	Number of plants.	Mosaic seedlings June 26.	Mosaic plants, Aug. 16.	
			Number.	Per cent.
Midwest.....	272	0	79	29
Elton.....	253	2	24	9
36847.....	236	0	1	0.4
Haberlandt.....	255	5	57	22
Wea.....	294	0	28	9
Lexington.....	352	1	8	2
Soysota.....	221	0	0	0
A. K.....	205	1	17	8
Black Eyebrow.....	160	1	21	13
Pinpu.....	209	0	7	3
Feldun.....	270	1	7	2
Dunfield.....	259	2	14	5
Ito San.....	250	0	4	2
Arlington.....	301	2	18	6
Manchu.....	166	1	12	7

In a small plot of Midwest soybeans planted in 1921 with seed from a field in which the disease occurred in 1920, 17 out of 423 seedlings, or 4 per cent, developed mosaic. A number of volunteer seedlings came up in 1921 in the field used in 1920 and one of these showed mosaic, indicating that the disease persisted over winter in the seed lying in the field.

Two rows were planted in 1921 with seed saved from mosaic Midwest plants in 1920 and out of the 156 seedlings, 14, or 9 per cent, showed mosaic. Among the progenies of six mosaic plants, 12 out of 65 seedlings, or 18 per cent, were mosaic.

In a plot planted in 1922 with seed saved from mosaic Midwest plants in 1921, 172 out of 993 seedlings, or 17 per cent, showed mosaic, while in another plot planted with seed from healthy plants, 590 seedlings came up and none were mosaic.

These results with seed from mosaic plants corroborate the writers' previous conclusion that a varying and usually rather low percentage of such seed carries the disease. Dickson (3, p. 83-86) obtained similar results in connection with the seed transmission of mosaic in *Trifolium pratense* L. and *Melilotus alba* Desr. Reddick and Stewart (8) found a varying but much higher percentage of transmission of bean mosaic through the seed, and Archibald (1, p. 62) found that 43 per cent of the seed from mosaic bean plants transmitted the disease. Doolittle and Gilbert (4) found that a low percentage of the seed from mosaic wild cucumber transmitted cucurbit mosaic, and Newhall (6) likewise found in the case of lettuce mosaic that a very low percentage of the seed transmitted the disease.

Seed saved from mosaic soybean plants of a number of varieties in the fall of 1921 were planted in the greenhouse the following winter with results as shown in Table II. The results of a similar test made with seed selected from mosaic plants in 1922 are shown in Table III.

TABLE II.—Seed transmission by different varieties, 1921

Variety.	Number of plants.	Number of seeds.	Number of seedlings.	Per cent germination.	Number of mosaic.	Per cent mosaic.
Midwest.....	10	676	535	79	121	23
Haberlandt.....	8	435	350	80	23	7
Black Eyebrow.....	1	31	23	74	8	35
Arlington.....	1	46	17	37	1	6
Feldun.....	1	151	122	88	4	3
Lexington.....	1	62	58	93	0	0
Dunfield.....	1	45	17	38	0	0
Manchu.....	1	64	27	42	0	0

TABLE III.—Seed transmission by different varieties, 1922-23

Tested.	Variety.	Number of plants.	Number of seedlings.	Number of mosaic.	Per cent mosaic.
In winter in greenhouse.	Midwest.....	10	114	11	10
	Haberlandt.....	8	97	7	7
	A. K.....	3	17	2	12
	Feldun.....	2	21	0	0
	Lexington.....	3	23	0	0
In summer of 1923 in field.....	Midwest.....	17	54	5	9
	Haberlandt.....	6	88	3	3
	A. K.....	2	18	0	0
	Lexington.....	2	20	0	0
	Manchu.....	2	18	0	0

The results shown in Tables II and III indicate that varieties differ somewhat in their ability to transmit mosaic. The Midwest, Haberlandt, Black Eyebrow, A. K., and Arlington varieties apparently transmit the disease more readily than Feldun, Manchu, Lexington and Dunfield.

Individual plants of the same variety also differ greatly in the extent to which the disease is transmitted to their progeny. In the progenies of six single selections (Midwest) planted in the field in 1921, the percentage of mosaic varied from 0 to 33 per cent.

In the progenies of the ten single plant selections from mosaic Midwest plants in Table II, the percentage of mosaic seedlings varied from 6 to 38 per cent, and among the eight Haberlandt progenies the percentage of mosaic varied from 0 to 16. In the progenies of the 19 Midwest plants recorded in Table III, mosaic was absent in eight and varied from 7 to 50 per cent in the others. Four of the eight Haberlandt progenies also showed no mosaic.

To determine whether this peculiar incidence of mosaic had any relation to the node at which the pods were borne, the seeds from nine mosaic Midwest and seven mosaic Haberlandt plants were harvested separately by nodes in 1921 and tested in the greenhouse. No particular relation was found between the percentage of mosaic seedlings and the location of the node at which the seed was borne. Numbering the bearing nodes from the top down, the second, third, fourth, and seventh yielded the most mosaic seedlings in the Midwest plants and the fourth node in the Haberlandt plants. Numbering the bearing nodes from the base upward, the fourth, seventh, and eighth yielded the most mosaic in the Midwest plants.

That the variation in percentage of seed transmission of mosaic was not dependent upon the date of infection of the parent plant is indicated by the fact that in the 19 Midwest parent plants recorded in Table III the mosaic was of seed origin, and yet an average of only 10 per cent of mosaic occurred in the progenies of these plants; in fact, in eight progenies no mosaic occurred. In 4 of the 8 Haberlandt parent plants the mosaic was also of seed origin and the progenies of these plants showed a lower average percentage of mosaic than the progenies of the other four plants.

The effect of age of seed upon the transmission of mosaic is of practical interest. The field tests noted above proved that the disease persisted in the seed from one season to the next. Among 1,105 Haberlandt seedlings grown in the greenhouse in December, 1922, from commercial seed 15 months old, 3.2 per cent were mosaic. To test the effect of an extra year's storage, commercial seed from two of the same lots tested in 1921 (Haberlandt and Arlington, Table I) was planted in the field in 1922. Of the 560 Haberlandt seedlings, 7 were mosaic and of the 629 Arlington seedlings 2 were mosaic. It thus appears that the disease was present in 2-year-old seed.

That 2-year-old seed may carry the mosaic disease was further demonstrated by greenhouse tests in the spring of 1923 with seed harvested from single mosaic plants in 1921. The results, as shown in Table IV, indicate that there was a considerable percentage of mosaic transmission in the old seed from mosaic plants in the Black Eyebrow and Midwest varieties and a low percentage in the Haberlandt variety.

TABLE IV.—*Presence of mosaic in seed stored 16 months*

Plant.	Variety.	Per cent germination.	Number of seedlings.	Number of mosaic.	Per cent mosaic.
1	Haberlandt.....	77	88	3	3.4
2	Black Eyebrow.....	35	21	3	14.3
3	Wea.....	75	40	0	0
4	Lexington.....	77	31	0	0
5	Arlington.....	28	19	0	0
6	.....do.....	14	14	0	0
7	Midwest.....	64	38	26	68.4
8	.....do.....	98	46	3	6.5
9	.....do.....	69	22	6	27.2

In the summer of 1923, seed from six mosaic Midwest plants collected in 1921 was planted in the field and 36 of the 208 seedlings, or 12 per cent, came up showing mosaic. These results indicate that the use of 2-year-old seed can not be recommended as a mosaic control measure.

In 1921 and 1922 a conspicuous brown mottling of the seed coat occurred rather generally and occasioned considerable concern among the growers of soybeans for seed. As yet no relationship has been established between this seed mottling and the mosaic disease. Mottled seeds have been produced by both healthy and mosaic plants and in germination tests a few mosaic seedlings were obtained from clean as well as from mottled seeds.

Ordinarily the seed from plants apparently free from mosaic has yielded only healthy seedlings. In a field plot in 1922 planted with such seed, no mosaic occurred among the 590 seedlings, while in another

plot planted with seed from mosaic plants, 172 out of 993, or 17 per cent, of the seedlings were mosaic. Owing to the drought of 1922, the field symptoms were not easily recognizable at the time the seed was collected, and among 42 single plant selections from supposedly healthy plants, 3 showed mosaic when tested.

It would seem, therefore, that seed selection from mosaic-free plants may be fairly effective, but not absolutely infallible, as a control measure.

#### SECONDARY SPREAD OF MOSAIC

The degree of spread of mosaic during the season of 1921 is shown in Table I in which the number of mosaic plants on August 16 is very greatly in excess of those noted on June 26. In the Midwest variety this secondary infection involved 29 per cent of the stand. In another plot of the same variety, 37 per cent of the stand became infected during the month between July 19 and August 19.

The spread of infection in 1922 was not so extensive as in 1921. In field plots, 14 per cent of the 2,174 Midwest plants, 16 per cent of the 487 Haberlandt plants, and 1 per cent of the 640 Arlington plants became infected during the season.

During 1923 the spread of infection was much more extensive than in the two preceding seasons. Among variety plots equally exposed to infection and showing no mosaic among the seedlings, the extent of secondary spread of mosaic is evidenced by the percentages of mosaic recorded August 7 to 14, as shown in Table V. The varieties Soysota and Virginia seemed to escape infection.

TABLE V.—*Secondary spread of mosaic, 1923*

Variety.	Number of plants.	Per cent mosaic.	Variety.	Number of plants.	Per cent mosaic.
Midwest.....	181	90	Brown.....	220	41
Manchu.....	24	79	Lexington.....	92	40
Haberlandt.....	76	73	Pinpu.....	50	38
Elton.....	28	64	Black Eyebrow.....	30	33
Feldun.....	43	63	36847.....	30	33
Dunfield.....	38	55	Mammoth Yellow.....	79	23
Wea.....	60	45	Tar Heel Black.....	253	22
Arlington.....	61	44	Wilson Black.....	329	14
Ito San.....	211	41	Soysota.....	163	2
A. K.....	39	41	Virginia.....	308	0.6

The agent of dissemination has not been determined. Aphids have not been noted to any extent on the soybeans. Leafhoppers and tarnished plant bugs have been found in abundance, but numerous tests with caged plants have failed to incriminate either of these insects.

#### EFFECT OF MOSAIC ON SEED GERMINATION

Dickson (3, p. 18-19) has shown that there is a reduction in the germinating power of seed due to the mosaic disease in the case of red clover and Canada field pea, and Cunningham (2, p. 27) states that seed from mosaic bean plants has a low germinating quality. In the greenhouse tests with seed from mosaic soybean plants presented in Table II there was 79 per cent germination of the 676 Midwest seeds planted,

80 per cent germination of the 435 Haberlandt seeds, and 88 per cent germination of the 151 Feldun seeds. Although lower percentages of germination occurred in some of the other varieties, there was no marked reduction of germinating power in the larger lots of seed nor was the germinability in any way correlated with the percentage of mosaic in the seedlings. The latter also holds true for older seed as shown in Table IV.

In field plots planted in 1921 with 370 seeds from mosaic plants only 61 per cent germinated. The relative germinability of the seed from mosaic and healthy plants grown in 1922 was tested in the greenhouse and the results, as shown in Table VI, indicate that mosaic had little if any influence on the germinating power of seed.

TABLE VI.—*Effect of mosaic on the germinating power of seed*

Variety.	Seed from healthy plants.			Seed from mosaic plants.			
	Number of plants.	Number of seeds.	Per cent germination.	Number of plants.	Number of seeds.	Per cent germination.	Per cent mosaic.
Haberlandt.....	8	176	74	8	137	71	7
A. K.....	2	45	78	3	21	81	12
Lexington.....	3	80	66	3	39	59	0
Feldun.....	2	51	63	2	39	54	0

#### EFFECT OF MOSAIC ON YIELD OF SEED

Reddick and Stewart (7) found that bean mosaic suppressed seed production, and Dickson (3, *p.* 18-19) has recorded a marked reduction in yield of seed due to mosaic in pea beans, broad beans, and red clover. As the writers (5) have previously reported, reduction in yield of seed is likewise one of the outstanding features of soybean mosaic. In the fall of 1921 a comparison was made of the yield of seed from mosaic and normal plants and the results, as presented in Table VII, show that mosaic caused a very serious reduction in yield, especially in the Haberlandt variety.

The comparative yield of healthy and diseased plants in the variety plots in 1922 is shown in Table VIII. The mosaic seedlings had been tagged so that it was possible to record whether the disease was of seed origin or the result of secondary infection during the season, and except in the Midwest variety, the mosaic was all of the latter type.

TABLE VII.—*Effect of mosaic on yield of seed, 1921*

Variety.	Healthy.		Mosaic.		
	Number of plants.	Average yield per plant in grams.	Number of plants.	Average yield per plant in grams.	Per cent reduction in yield.
Midwest.....	24	14.59	27	9.58	34
Haberlandt.....	5	41.57	9	10.96	76
Arlington.....	3	7.65	3	4.60	40
Lexington.....	2	12.01	2	4.82	60

TABLE VIII.—Effect of mosaic on yield of seed, 1922

Variety.	Condition of plants.	Number of plants.	Average number seeds per plant.	Average yield per plant.	Reduction in yield.	Average weight of a seed.
				Gms.	Per cent.	Gms.
Midwest.....	Healthy.....	28	18.0	1.85	.....	0.103
	Mosaic (seed origin)...	95	4.4	.46	75	.105
Haberlandt....	Healthy.....	22	22.0	3.01	.....	.137
	Mosaic.....	13	14.7	2.04	32	.138
Feldun.....	Healthy.....	6	21.6	3.25	.....	.141
	Mosaic.....	5	14.0	1.77	45	.126
Lexington.....	Healthy.....	7	22.8	2.00	.....	.151
	Mosaic.....	6	12.5	.93	53	.075
Dunfield.....	Healthy.....	4	35.7	4.65	.....	.130
	Mosaic.....	4	13.2	1.47	68	.111
Arlington.....	Healthy.....	6	40.8	2.56	.....	.062
	Mosaic.....	4	6.7	.23	93	.033
Manchu.....	Healthy.....	3	19.3	2.40	.....	.124
	Mosaic.....	3	10.3	1.10	54	.106
A. K. ....	Healthy.....	9	27.2	3.75	.....	.137
	Mosaic.....	8	9.6	1.14	69	.118

Owing to the drought, all of the yields were very low in 1922 as compared with 1921. However, the results in Table VIII show that mosaic caused heavy losses in all of the varieties. The loss in the Midwest variety, of which the greatest number of mosaic plants were available, amounted to 75 per cent. All of these Midwest plants represented mosaic of seed origin and 37 of the 95 bore no seeds whatever. In the Haberlandt variety, however, the average yield of seven plants with mosaic of seed origin, not recorded in Table VIII, was only 14 per cent less than that of the healthy plants.

It will be noted that in general the loss was due to the fewer number of seeds per plant rather than to the smaller size of the seeds, although in the last six varieties the seeds from mosaic plants were considerably smaller, especially in the Lexington and Arlington varieties.

#### SUMMARY

No host for soybean mosaic has been found other than the soybean itself.

Varieties of soybean seem to differ somewhat in susceptibility. Midwest has proved very susceptible; Soysota and Virginia have shown a tendency to escape infection.

Generally about 10 to 25 per cent of the seed from mosaic plants produced mosaic seedlings.

Varieties seem to differ in their ability to transmit mosaic through the seed. Midwest, Haberlandt, Black Eyebrow, A. K., and Arlington readily transmitted the disease.

Marked differences occurred in the percentages of mosaic in the progenies of individual mosaic plants of the same variety.

The transmission of mosaic seems to bear little or no relation to the location of the node at which the seed was produced nor to the date of infection of the parent plant.

The disease has been found in 2-year-old seed saved from mosaic plants.

A conspicuous brown mottling of the seed coat has not been correlated with mosaic.

Seed selected from mosaic-free plants gave rise to mosaic-free seedlings.

A considerable spread of mosaic occurred during the growing season. This secondary spread was more extensive in some seasons than in others.

Apparently the disease did not materially lower the percentage of seed germination.

Mosaic reduced the yield of seed 30 to 75 per cent. The number of seeds per plant was greatly reduced; in fact, plants with mosaic of seed origin frequently bore no seeds whatever.

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# INSECTICIDAL EFFECT OF COLD STORAGE ON BEAN WEEVILS<sup>1</sup>

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## INTRODUCTION

The present paper is a report on the insecticidal efficiency of certain commercial cold-storage temperatures upon our two most destructive insect pests of stored legumes, *Bruchus obtectus* Say, the common bean weevil, and *Bruchus quadrimaculatus* Fab., the four-spotted bean weevil. The effectiveness of cold in protecting beans, peas, and cowpeas against weevil attack has long been recognized, but relatively little attention has been given by investigators to the use of low temperatures for killing weevils which are present in stored legumes.

Duvel (2)<sup>3</sup> found that refrigeration at 32° to 34° F. was a perfect protection. Krall (4) concluded that eggs as well as all stages of the cowpea weevil, *Bruchus chinensis* L., can be killed by storage at 32° F. or colder (duration of exposure not given). Garman (3) stated that a temperature of 32° F. suspends the activity of the weevils and in time may kill them, although short exposures to this temperature apparently have no killing effect. He also concluded that all stages including eggs may be killed by one night's exposure to zero weather. The foregoing writers found that the germinating power of the seeds is not injured by refrigeration, even (2) for 20 months. Back and Duckett (7), Severin (5), and others stated that little or no development takes place at or below 50° F.; and Garman (3), speaking of temperatures of 35° to 40° F., wrote that "even these temperatures stop the work of the insects, though they will not entirely destroy them."

The present report summarizes experiments with *Bruchus obtectus* in California pink beans (*Phaseolus vulgaris*) and with *B. quadrimaculatus* in black-eye cowpeas or beans (*Vigna sinensis*). The results of work with the two species are here considered separately.

## COLD-STORAGE EXPERIMENTS WITH BRUCHUS OBTECTUS

### SCOPE AND METHOD

These experiments included the refrigeration of 92,484 California pink beans infested with an estimated total of 114,200 larvæ, pupæ, and adults. The temperatures tested, 32° and 36° F., were obtained in a modern cold-storage plant at Stockton, Calif., where inspection of the rooms by employees every two hours served to keep the desired temperatures fairly constant. The term "constant temperature" is used here in the commercial sense, and it is assumed that there were occasional

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<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 105.

fluctuations of a few degrees. Cotton sample bags were filled with from 3,000 to 5,000 infested beans each, obtained from commercial storage, and these were removed at intervals and mailed to the laboratory at Alhambra, Los Angeles County, Calif. The time spent in transit was sufficient for the insects not killed to become active.

A short time after they were received at the laboratory the beans were counted and a sample of 200 taken at random from each sack. These were dissected and all weevils found—larvæ, pupæ, and unemerged adults, both alive and dead—were recorded. Subsequent examinations of the sacks of beans were made at various times.

#### RESULTS WITH STORAGE AT 32° F.

Eleven samples of beans were exposed at 32° F. for periods ranging from 7 to 61 days. When the sacks were opened numerous emerged adult weevils were found. Among the 43,281 beans in the 11 samples, 5,649 adults were counted, 5,272 dead and 377 alive. No live adults occurred in samples refrigerated for 46, 56, or 61 days. Samples exposed for 7, 14, 22, 28, and 31 days contained sufficient live emerged weevils to reinfest the seeds. Although the condition of the emerged insects found is a rough index of the effect of the various exposures, it is necessary to refer to the results of subsequent examinations in order to ascertain the ability of the insects which survived within the beans to perpetuate the infestation.

At the time of the first examination, all emerged weevils were removed from the sacks. Reexaminations showed that some of the larvæ, pupæ, and adults which were alive within the beans at that time were able to emerge, following exposure to laboratory temperatures favorable to growth and reproduction. Heavy reproduction took place in beans refrigerated for 7 days, and live weevils were found in sacks subjected to 14 and 28 days of cold storage at 32° F.

The living weevils taken from a number of the samples at the first examination were placed under conditions favorable for reproduction, and it was found that adults from samples refrigerated 14 days or more were very weak and seemed to have lost much of their vitality; those from beans stored 22 days or longer failed to deposit eggs. Six other samples, not included in the tabulations given in this report, were exposed to 32° F. for 66 days and all emerged weevils were found to be dead. These samples were estimated to contain 20,500 beans and 43,700 weevils. A later inspection showed that this exposure had completely killed the infestation.

Table I gives the results of the dissection of 200 beans from each of the refrigerated sacks. A total of 2,200 beans dissected to represent the average condition of the 11 samples contained 2,360 weevils; 1,324 (56 per cent) of them were larvæ; 630 (27 per cent) were pupæ, and 406 (17 per cent) were adults which had not emerged from the pupal cells. The percentages of all forms killed after different periods of refrigeration are seen to be not very consistent with the increasing mortality to be expected with increasing exposure to cold. The samples were put in storage at two different times of year, November and February, and this seems to be related to the resistance of the insects, those subjected to refrigeration in November being less resistant than the others. The samples in which the mortality was 69.54, 71.48, and 97.20 per cent were

taken in November from a warehouse where the temperature was about 65° F., while those showing mortalities of 40.43, 52.17, 68.86, 80.45, and 85.13 per cent went into cold storage from a lower temperature in February.

In Table I the perfect control obtained after 56 days at 32° F. is shown. Forty-six days' exposure did not kill all the weevils within the beans, although, as previously stated, no live emerged adults were found.

TABLE I.—Effect of a constant temperature of 32° F. on *Bruchus obtectus*

Number of days in storage.	Larvæ.			Pupæ.			Unemerged adults.			Mortality, all forms.
	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	
			<i>Per cent.</i>			<i>Per cent.</i>			<i>Per cent.</i>	<i>Per cent.</i>
7	56	154	73.73	25	55	68.75	25	33	56.90	69.54
14	15	91	85.85	30	52	63.41	38	65	63.11	71.48
14	14	6	30.00	3	6	66.66	11	7	38.89	40.43
22	16	20	64.44	11	4	26.66	6	3	33.33	52.17
28	1	284	99.65	1	152	99.35	12	51	80.95	97.20
31	21	32	60.38	6	22	78.57	6	19	76.00	68.86
37	14	64	82.05	11	34	75.56	1	9	90.00	80.45
46	5	36	87.80	3	16	84.21	3	11	78.57	85.13
56	0	185	100.00	0	82	100.00	0	46	100.00	100.00
56	0	265	100.00	0	91	100.00	0	29	100.00	100.00
61	0	36	100.00	0	26	100.00	0	31	100.00	100.00
Total...	142	1,182	.....	90	540	.....	102	304	.....	.....

#### RESULTS WITH STORAGE AT 36° F.

Seven samples of beans were refrigerated at 36° F. for periods ranging from 14 to 66 days. At the first examination of the samples, which contained 28,703 beans, 2,199 weevils were found emerged—2,092 were dead and 107 alive. Samples exposed for 14, 22, 31, 37, and 46 days contained a sufficient number of live adults to reinfest the seeds. No live adults occurred among beans stored for 61 and 66 days.

Subsequent examination of these samples showed that new emergence had taken place from beans stored for 14, 22, 31, and 37 days, and that none had occurred in the 46 and 61 day samples; but one live adult was found to have emerged from beans stored 66 days at 36° F. As shown in Table II, 200 beans from this sample contained no live larvæ, pupæ, or adults, and the presence of one live adult in the sack 3 months after removal from cold storage is regarded as accidental.

The live weevils removed from samples at the time of the first examination were kept under favorable conditions to test their powers of reproduction. A few eggs were laid by females refrigerated for 32 days or longer, but all of these failed to develop. This fact tends to minimize the importance of the presence of a few feeble survivors.

Table II gives the results of dissecting 200 beans from each sample stored at 36° F., and shows (as with 32° F.) that the length of exposure which killed all emerged adults was not sufficient to give perfect control of forms within the beans. As previously stated, 61 days' refrigeration killed all emerged weevils, although some survived within the beans. The

1,400 beans dissected contained 792 weevils, and the larvæ showed a slightly greater percentage of mortality than pupæ and a considerably greater percentage of mortality than unemerged adults. Judging from the conditions found in the dissected beans, 66 days at 36° F. killed all weevils.

TABLE II.—*Effect of a constant temperature of 36° F. on Bruchus obtectus*

Number of days in storage.	Larvæ.			Pupæ.			Unemerged adults.			Mortality, all forms.
	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	Number alive.	Number dead.	Mortality.	
			<i>Per cent.</i>			<i>Per cent.</i>			<i>Per cent.</i>	<i>Per cent.</i>
14	4	22	84. 61	11	21	65. 62	7	17	70. 83	73. 17
22	25	28	52. 83	4	18	81. 82	7	12	63. 58	61. 70
31	11	17	60. 71	3	27	90. 00	9	19	67. 86	73. 25
37	6	21	77. 78	8	13	61. 90	10	13	56. 52	66. 20
46	7	24	77. 42	2	18	90. 00	7	17	70. 83	78. 67
61	0	20	100. 00	1	25	96. 15	5	23	82. 14	91. 89
66	0	220	100. 00	0	58	100. 00	0	32	100. 00	100. 00
Total...	53	352	.....	29	180	.....	45	133	.....	.....

## COLD STORAGE EXPERIMENTS WITH BRUCHUS QUADRIMACULATUS

### SCOPE AND METHOD

The resistance to cold of the eggs of the four-spotted bean weevil was tested by refrigerating 40,000 eggs (estimated) on about 6,000 black-eye cowpeas, with controls estimated at 13,300 eggs on 2,000 black-eye cowpeas. The eggs were deposited during a period of a few days before the seeds were placed in refrigeration, and had not begun to hatch at that time. The cowpeas on which the eggs had been deposited were placed in four thin cotton sample bags, each containing about 4,000 seeds.

The effect of cold upon the other stages of this insect was investigated by the cold storage of 12,000 black-eye cowpeas containing an estimated total of 36,000 weevils, with a control of 4,000 seeds infested to the same degree. Four sample bags were filled with the weevily cowpeas, each bag containing about 4,000.

One bag of cowpeas bearing eggs and one containing weevily cowpeas were left in the laboratory as controls, and similar pairs of bags were placed in each of the temperatures tested: 20°, 32°, and 39° F. The refrigerated rooms were in a modern cold-storage plant at Pasadena, Calif., where the temperatures were not permitted to vary more than 1° F. either way.

The weevily cowpeas used in the trials were taken from a 100-pound bag which was "heating" because of heavy infestation; the temperature within the bag varied from 95° to 100° F. (14 to 35° F. above room temperatures when the readings were made). About 30 minutes elapsed between the time the samples were taken from the laboratory and their storage in the cold rooms.

The cowpeas were examined at intervals, at which times samples of 50 or of 100 seeds were taken from each bag and transferred to the laboratory, where they were kept at temperatures favorable to growth and reproduction.

## EFFECT OF 32 DAYS' REFRIGERATION ON SUBSEQUENT EMERGENCE OF ADULTS

The emergence of adults from three of these small lots after 32 days' exposure to 20°, 32°, and 39° F. is shown in Table III.

TABLE III.—Emergence of *Bruchus quadrimaculatus* from black-eye cowpeas refrigerated 32 days

Temperature of refrigeration.	Interval between removal from cold storage and examination.	Number of weevils emerged.			Temperature of refrigeration.	Interval between removal from cold storage and examination.	Number of weevils emerged.		
		Male.	Female.	Total.			Male.	Female.	Total.
°F.	Days.				°F.	Days.			
20.....	7	0	0	0	39.....	7	2	1	3
20.....	16	0	0	0	39.....	16	0	0	0
20.....	23	0	0	0	39.....	23	2	2	4
20.....	31	0	0	0	39.....	31	0	0	0
20.....	52	0	0	0	39.....	52	0	0	0
20.....	75	0	0	0	39.....	75	0	0	0
32.....	7	0	0	0	Control	7	7	6	13
32.....	16	0	0	0	Do.	16	10	4	14
32.....	23	0	0	0	Do.	23	7	8	15
32.....	31	0	0	0	Do.	31	25	14	39
32.....	52	0	0	0	Do.	52	21	19	40
32.....	75	0	0	0	Do.	75	29	21	50

The data in Table III indicate that 32 days of refrigeration at 20° or 32° F. will kill all forms, but that a longer time at 39° is necessary to kill all infestation within the cowpeas.

## EFFECT OF LOW TEMPERATURES ON IMMATURE STAGES OF WEEVILS

These low temperatures cause a dark spot to appear in the middle of the dorsal region of the larvæ. As this spot enlarges it becomes surrounded by a lighter band, and when approximately the middle one-fourth of the dorsal region becomes discolored the tissues beneath the integument soften and the larva dies. The same signs of injury can be observed in young pupæ.

## EFFECT OF REFRIGERATION ON LARVÆ, PUPÆ, AND UNEMERGED ADULTS

As previously noted, a small lot of cowpeas was removed from each bag in refrigeration at the time of each examination. Some of these were dissected a few days after refrigeration; others were kept as long as seven months. Table IV gives the data obtained by the dissection of these samples. The dissection of 13 cowpeas four days after removing them from refrigeration at 20° F. for 11 days showed 20 per cent of the weevils to be alive, but the other 37 cowpeas of the same sample subsequently produced no adults. This indicates that although 20° F. does not kill all the unemerged weevils immediately, it injures them to such an extent that they die before emerging.

The indications of the foregoing data are that weevils emerge from cowpeas refrigerated at 39° F. for 11 and 18 days, respectively, but not after exposure to this temperature for 25 days (though this is

not in agreement with the results of 32 days' exposure to 39° F., as shown in Table III). Eleven days' exposure to 32° or to 20° F. gave a complete kill, judging from the conditions found in the samples, while 18 days' exposure to 39° F. did not kill the infestation.

TABLE IV.—Results of dissection of refrigerated cowpeas

Temperature.	Duration of refrigeration.	Interval between removal from refrigeration and dissection.	Number of cowpeas dissected.	Stages dissected from cowpeas.								Total.	Dead.
				Adults.			Pupæ.		Larvæ.				
				Alive.	Dead.	Emerged before dissection.	Alive.	Dead.	Alive.	Dead.			
°F.	Days.	Days.										Per cent.	
20.....	4	10	50	3	7	0	7	8	9	27	61	69	
20.....	11	4	13	0	6	0	2	5	1	1	15	80	
		Months.											
20.....	11	7	37	0	8	0	0	10	0	100	118	100	
20.....	18	7	50	0	17	0	0	33	0	106	156	100	
20.....	25	7	50	0	20	0	0	40	0	89	149	100	
20.....	32	6	100	0	34	0	0	82	0	184	300	100	
32.....	11	6	50	0	15	0	0	29	0	87	131	100	
32.....	18	6	50	0	19	0	0	52	0	94	165	100	
32.....	25	6	50	0	23	0	0	65	0	72	160	100	
32.....	32	6	100	0	28	0	0	83	0	236	347	100	
39.....	11	6	50	0	12	8	0	37	0	97	154	95	
39.....	18	6	50	0	22	4	0	42	0	88	156	97	
39.....	25	6	50	0	21	0	0	47	0	80	148	100	
39.....	32	6	100	0	18	0	0	70	0	261	349	100	
Control.....		4	100	3	9	48	93	17	11	121	302	49	

## EFFECT OF REFRIGERATION ON THE VIABILITY OF THE EGGS

Table V shows that four days' refrigeration at 32° or 20° F. is sufficient to prevent all eggs from hatching; a temperature of 39° F. for the same length of time, however, leaves some eggs viable. Of the eggs removed from a temperature of 39° F. after four days' refrigeration and exposed to favorable temperatures, three hatched and one of the three larvæ developed and emerged.

TABLE V.—Effect of refrigeration on the viability of eggs of *Bruchus quadrimaculatus*

Duration of refrigeration.	Temperature.	Number of beans removed.	Number of eggs.	Number hatched.	Duration of refrigeration.	Temperature.	Number of beans removed.	Number of eggs.	Number hatched.
Days.	°F.				Days.	°F.			
4.....	20	50	a	0	25.....	32	50	a	0
11.....	20	50	a	0	32.....	32	100	656	0
18.....	20	50	a	0	4.....	39	50	a	b 3
25.....	20	50	a	0	11.....	39	50	a	0
32.....	20	100	664	0	18.....	39	50	a	0
4.....	32	50	a	0	25.....	39	50	a	0
11.....	32	50	a	0	32.....	39	100	697	0
18.....	32	50	a	0	Control.....		100	662	456

a Not counted.

b Produced 1 adult.

A comparison of Tables IV and V shows that the eggs are more easily killed by low temperatures than the more advanced stages.

#### SUMMARY

##### BRUCHUS OBTECTUS

Exposure of larvæ, pupæ, and adults of *Bruchus obtectus*, infesting California pink beans, to 32° F. for 56 days or to 36° F. for 66 days gave very satisfactory control. Considerably shorter periods of exposure rendered the surviving adults incapable of reproduction.

##### BRUCHUS QUADRICACULATUS

A temperature of 39° F. for 32 days was insufficient to kill all stages within black-eye cowpeas. All stages were killed when subjected to 32° F. or colder for 32 days.

The eggs were killed by four days' refrigeration at 32° or 20° F., but a longer time was necessary to kill them at 39°. They were more susceptible to cold than the other stages.

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# THE EFFECT OF RUST INFECTION UPON THE WATER REQUIREMENT OF WHEAT<sup>1</sup>

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## INTRODUCTION

During years of severe rust epidemics, such as those which the spring wheat sections of the northern Mississippi Valley and the prairie Provinces of Canada experienced in 1904 and 1916, and in other years, attempts have been made to estimate the loss suffered by the wheat crop as a direct result of the rust. Such attempts usually have been based upon the comparative yields in rust years and the average yield over a number of years in which rust was absent or not severe. It has not been possible to compare directly, on a large scale, the yields of rusted and rust-free fields where variety, soil type, and growing conditions are similar, because during rust epiphytotics nearly all fields are too generally affected. Furthermore, as a characteristic sequence of weather conditions usually occurs in rust years, it has been impossible wholly to differentiate the effect of rust from that of climate.

A scientific basis for apportioning the responsibility between the rust and the departures from normal weather is attainable only through the artificial maintenance of rusted and rust-free plots in which all circumstances are otherwise similar, and by noting the effect on reduction of yield as the several growth factors are varied. The factors which most require this sort of investigation are those associated with fertilization and moisture content of the soil, but an examination of the literature of the cereal rusts affords only meager information in respect to each.

As a beginning in this line of investigation, experiments were planned to determine the effect of rust infection upon the water requirement of the wheat plant and the influence of different conditions of nutrition on the water relations. It was believed that such data might be useful as a basis for separating the factors of climate and parasitism in the reduced yield of rusted wheat, particularly as regards the water requirement. It was desired further to compare the results of these cultures with those of soil-grown greenhouse and field plants in respect to the relation of fertilization to rust infection. Accordingly, this paper is divided into two parts, one of which deals with the effect of rust infection upon the water requirement, and the other with the influence of certain mineral nutrients on susceptibility to rust and injury from it.

<sup>1</sup> Accepted by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, July, 1922. Received for publication by the Journal of Agricultural Research Oct. 16, 1923. Published with the approval of the Director, as Paper 416 of the Journal Series, Minnesota Agricultural Experiment Station.

The investigation herein reported was conducted cooperatively between the Office of Cereal Investigations, U. S. D. A., and the Department of Agriculture of the University of Minnesota. It was undertaken as a part of the general project on the effect of soil nutrients on the development of stem rust and orange leaf rust of wheat.

## EFFECT OF RUST ON WATER REQUIREMENT

REVIEW OF LITERATURE DEALING WITH EFFECT OF PARASITIC INFECTION  
ON THE WATER REQUIREMENT OF PLANTS

The literature of transpiration which relates to the effect of parasitic infection on the water use of the host is not extensive. Burgerstein (6)<sup>2</sup> cites the work of Müller-Thurgau (11) who, using the cobalt-chlorid paper method, measured the alteration in transpiration rate induced by infection of pear and apple leaves by scab and mildew fungi. Leaves infected by *Fusicladium* showed, in the diseased areas, increased transpiration as compared with adjacent healthy portions, especially if the upper leaf surface bore the lesion. *Sphaerella*, on the other hand, caused no marked change, while the lesions produced on grape leaves by *Peronospora viticola* (sic) lost almost no water at all, due to stoppage of the stomata by the conidiophores.

Blodgett (2) observed that a shoot of *Rubus*, heavily infected by *Gymnoconia*, took up nearly twice as much water as a comparable healthy branch, yet its leaves wilted while those of the latter remained turgid.

Reed and Cooley (13, 14) measured the water loss from healthy apple leaves as compared with those infected by *Gymnosporangium*, making the determinations on leaves *in situ* and using an apparatus in which the transpired water could be absorbed and weighed. Under all conditions the amount of water transpired by infected leaves was less than that by healthy ones, the reduction being about 25 per cent. This was attributed to hypertrophy of the spongy parenchyma, reduction of intercellular spaces and the filling of these by fungus hyphae, and to the absence of stomata on the aecial cushions. At an early stage of infection the transpiration of affected leaves was not much less than that of sound ones. The reduction of transpiration was greatest at full development of the aecial cushions together with spore liberation. A rise in the rate of transpiration of rusted leaves occurred later when the lesions were surrounded by extensive areas of dead tissue. The possibility of an intoxicating effect on the host cells produced by fungous excretory products also was suggested.

Rust infection of cereals was found by Humphrey<sup>3</sup> and by Weaver (22) to cause accelerated transpiration. Weaver grew wheat, rye, barley, oats, and corn infected by stem and leaf rusts and compared the water loss under a variety of light and temperature conditions with that from uninfected controls. The amount of transpiration was determined by weighing the sealed pots at intervals and was expressed in grams per square centimeter of leaf surface. The rate was consistently higher in the rusted plants, the excess being greatest under environmental conditions favoring rapid water loss. A quantitative relation between acceleration of rate and area of the pustules was demonstrated. So slight an infection of oat leaves as involved only 0.5 per cent of the leaf area caused the transpiration rate to rise by 37 per cent. On the other hand dicotyledonous plants such as *Xanthium*, *Helianthus*, and *Dianthus*, when attacked by rust, either lost less water by transpiration than uninfected ones, or showed a slight acceleration when the pustules appeared, followed by a reduction if infection remained restricted to the original leaves.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 117-118.

<sup>3</sup> HUMPHREY, H. B. Unpublished data cited by J. E. Weaver (22).

Reynolds (15) had previously described the effect of infection of apple leaves by *Gymnosporangium*, and of Xanthium by *Puccinia xanthii*, upon the tissue and cell morphology of the host. In general, the rusts were found to produce hypertrophic changes, but the separation from the mesophyll and the rupture of the epidermis at pustule formation exposed uncultivated cells to the drying effects of the atmosphere, resulting in atrophy. In Xanthium the loose organization of the mesophyll in healthy leaves is altered in rusted ones to a compact tissue composed of mycelium and empty host cells. The work of Ward, Evans, and others showed that in the grass rusts atrophic changes in the host cells do not appear until extensive development of the rust mycelium prior to pustule formation. In the case of resistant hosts, Stakman (17, 18) found that disorganization of the chloroplasts followed by death and disintegration of the cells occurs in proximity to the invading germ tube.

Weaver rightly considered that rupture of the epidermis and death of the chlorenchyma cells in the infected area produce the marked acceleration in transpiration evident at pustule formation. He believed that toxic excretory products of the parasite also might stimulate transpiration, but are of slight importance in the cereal rusts. It was pointed out that transpiration may not always be an index of growth, for water loss from infected plants is excessive and bears no relation to the production of new substance. He did not determine the correlative transpiration, or amount of water transpired per unit of weight. The question of a sustained accelerating effect of rust infection on transpiration was raised, and the statement made that after the first pustules erupted no further change in rate was evident, the accelerated rate being maintained by the appearance of new pustules.

Dufrenoy (8) has given an interesting summary of the effects produced by a number of parasites and epiphytes on the transpiration of their hosts. Chlorotic areas of variegated leaves, and such as surround points of infection, transpire less rapidly than the green portions; the red-pigmented areas surrounding lesions caused by *Polystigma rubra* on plum leaves transpire more rapidly, contrary to the behavior of anthocyanin-containing cells generally. A number of parasites accelerate transpiration, owing to localized necrosis, rupture of the epidermis by fruiting bodies, or the production of overgrowths, although some witches' brooms appear to be xerophytic modifications. As the same effect sometimes is shown by epiphytes, changes in permeability of the host cells are involved also. A table is given which shows that among diverse hosts and parasites the transpiration of infected, as compared with healthy, leaves and parts of organs varies in a ratio of 0.5 to 1 to 100 to 1. The results were obtained by the use of hygroscopic paper.

It is to be noted that in none of the experimental demonstrations of acceleration of transpiration rate produced by parasitic infection which are reviewed above has the ultimate effect on the water economy of the host been determined. This would seem to be the most logical criterion of the general effect on the plant's water relations caused by parasitic attack.

Burkholder (7) has taken this into account in his study of the reduction in yield of beans resulting from infection by *Fusarium* rootrot. Infected plants gave only about half the dry weight of seed yielded by controls and transpired less water, the reduction amounting to about 50 per cent at soil temperatures of 18° and 26° C. Transpiration thus appeared to be an approximate index of growth.

Wimmer (23) showed that the water requirement of sugar beets and celery is increased by nematode infection, and believed this to be due to the slower growth of such plants. It is well known that the "compound interest law of plant growth" (1) falls far short of realization when growth is abnormally slow, and lowered water economy results also.

That parasitic fungi may affect the water relations of the host in other ways than by mechanical rupture of the tissues which normally protect the transpiring tissues, or by causing less economical use of water owing to retarded growth, is evident from the work of Haskell (9), who showed that in *Fusarium* wilt of potatoes, either the destruction of roots or the production of toxic excretory products by the fungus may be responsible for wilting of the plant, but mechanical stoppage of the vessels does not occur. The effect on water requirement is unknown.

Fungous infection may result either in acceleration or diminution of transpiration. The character and intensity of the effect are determined by the mechanical rupture of protective tissues, by local necrosis of the epidermis or mesophyll, or by toxic excretions, all of which result in accelerated water loss. On the other hand, transpiration may be diminished by hypertrophy of the chlorenchyma, reduction of air spaces, and number of stomata, by production of inhibitory excretions, by alteration of the osmotic relations of the infected cells and by injury to the water-absorbing parts.

The final or aggregate effect of rust infection on the water requirement of cereals has not been adequately determined. Since Weaver's results were secured with seedlings for a short growth period, it seemed that further work was necessary to obtain evidence in regard to the following three questions:

Is the transpiration of rusted wheat plants sustainedly greater than that of healthy plants throughout the growth period?

Is the amount of water transpired for the production of a unit weight of plant substance greater in rusted than in healthy plants, and are grain and straw production affected in the same way?

Is the effect on water requirement different in the case of infection by leaf rust and by stem rust, and is this effect related to differences in character and sizes of pustules?

#### EXPERIMENTAL METHODS

Marquis wheat was selected for the experiment, together with several strains of stem rust which produce large confluent pustules and no chlorosis on this host, and a mixture of collections of leaf rust to which this variety was readily susceptible. The plants were grown in Wausau quartz sand of a grade having a moisture equivalent of 3 per cent and a maximum water capacity of about 12 per cent. Cocks of one-half gallon capacity, provided with a drain at the bottom, served as pots. Two thousand eight hundred gm. of air-dry soil were placed in each. Three hundred cc. of nutrient solution were added, thus bringing the water content close to the maximum capacity, and one plant was set in each cock. The soil moisture content was maintained by weighing the cocks first at weekly and then at three-day intervals, and water was added to restore the base weight. The cocks were sealed with wax so that water loss was restricted to the plant. Although in the latter part of the growth period transpiration became very great, owing to the high diurnal temperatures in the greenhouse, there was no indication of

wilting at any time; and since the soil water-content was brought back to maximum at three-day intervals, it is believed that these plants made their growth under very favorable conditions of soil moisture. Therefore the reduced yield shown by infected plants should be attributed to the rust and not to lack of sufficient moisture for normal growth at any stage of development.

Mineral nutrients were supplied in solution as stated. The composition of the different nutrient solutions used is shown in Table I. As a basic solution Shive's (16)  $R_2C_2$  was used containing  $Ca(NO_3)_2$ ,  $KH_2PO_4$  and  $MgSO_4$  in the gram-molecular proportions 0.0078, 0.0108, and 0.0030,<sup>4</sup> respectively, and 5 cc. of ferric phosphate solution [Trottingham (19)] was added to each liter. Stock solutions of the salts were made up in half or quarter molecular concentration, and diluted at the time of using. The complete nutrient solution was made up fresh for each renewal.

To the basic nutrient solution, nitrogen as  $NO_3$ , phosphorus as  $PO_4$ , and potassium as chlorid, were added in two concentrations to form six modified solutions, the added salt in each case being one which did not introduce any cation under test. (See Table I.) The concentrations of added ions were of the order that would result from the application of 300 pounds of sodium nitrate, 420 pounds of sodium biphosphate, and 260 pounds of potassium chlorid per acre, for the lower concentration, and double these quantities for the greater concentration, assuming that all the added salts would be retained in the surface foot.

TABLE I.—Composition in grams and gram-molecules per liter of the nutrient solutions used in 11 different cultures

Component salts.	OO	OA	OB	IA	IB	IIA	IIB	IIIA	IIIB	IVB	VB
$Ca(NO_3)_2$ :											
Gm.....	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279	1.279
Gm. mols.....	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078
$NaNO_3$ :											
Gm.....				0.723	1.454						
Gm. mols.....				0.0085	0.0171						
$KH_2PO_4$ :											
Gm.....	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471	1.471
Gm. mols.....	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108	0.0108
$NaH_2PO_4$ :											
Gm.....						1.020	2.050				
Gm. mols.....						0.0085	0.0171				
$MgSO_4$ :											
Gm.....	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361	0.361
Gm. mols.....	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030	0.0030
$MgCl_2$ :											
Gm.....											1.629
Gm. mols.....											0.0171
$CaCl_2$ :											
Gm.....										1.896	
Gm. mols.....										0.0171	
KCl:											
Gm.....								0.634	1.274		
Gm. mols.....								0.0085	0.0171		
NaCl:											
Gm.....		0.500	1.000								
Gm. mols.....		0.0085	0.0171								

A further modification of the basic solution was prepared by adding NaCl in the same molecular proportions as in the case of those salts of direct nutrient value in order to produce an osmotically equivalent<sup>5</sup>

<sup>4</sup> Shive's solution actually contained 0.0020 gm. mols. of  $MgSO_4$ , but the other of his two best solutions for wheat contained 0.0030; hence this slight change was made.

<sup>5</sup> Since these solutions were all prepared in gram-molecular rather than equivalent-molecular proportions, and since dissociation varies in the different salts, they are only approximately isosmotic. This is not considered to be significant as the solutions were employed in this experiment.

modification of the basic solution which contained no added ions of direct value. Two further modifications were prepared, one containing 0.0171 gram-molecules of  $\text{CaCl}_2$  per liter, the other an equivalent quantity of  $\text{MgCl}_2$ . Eleven different solutions were used in all, each applied to four crocks.

The nutrient solution was renewed at intervals of three weeks by opening the drain at the bottom of the crock and flushing the sand with fresh solution. When the solution ceased to drain from the openings the crocks were weighed for a new base weight. As in all cases the amount of solution retained approximated the maximum water capacity of the soil, the successive base weights were in fairly close agreement.

The plants were grown from November to June. The long growth period and slow development during the winter resulted in a high water requirement as compared with field-grown plants. It was March before heading began and the end of May before the majority of the plants were ripe. It appears that length of day is an important factor in conditioning the time of maturity of greenhouse-grown wheat. It was necessary to cut the plants before all of them, particularly those in the excess N and K series, were fully ripe: the data from these are not considered in the summary, but a majority of the plants had plump hard grain at this time. The yield was rather below that of field-grown plants, the average for the three series of stem rust, leaf rust, and control plants translated into terms of bushels per acre being 9.6, 9.1, and 11.5, respectively. Certain cultures (four crocks), however, yielded at the rate of 20 to 25 bushels per acre and the best individual plant on a similar basis yielded 34 bushels per acre.

Three series of crocks were treated with each of the 11 culture solutions. One of these was left uninoculated as a control; in the second and third infection by stem rust and by leaf rust, respectively, was produced by artificial inoculation. Leaf-rust infection was readily obtained by atomizing the plants with a water suspension of urediniospores, but this method was unsuccessful with stem rust. With the latter, initial infections were produced by moistening the leaves and then applying dry urediniospores, following which steam was allowed to escape into the house for 48 hours while the ventilator remained open. In this way a nearly saturated atmosphere and dense fog could be maintained for two days at a time, resulting in copious precipitation of dew on the foliage. This process was repeated at monthly intervals to simulate the succession of new infections that occurs in the field. By these means a severe epiphytotic of stem rust was maintained, and a lighter one of leaf rust, during more than half of the growth period. In each case fairly uniform infection was produced on the replicate crocks of a culture, although some diversity in this respect could not be avoided.

The quantity of rust was estimated at the time of heading according to the rust scale of the Office of Cereal Investigations; but it is to be noted that at this time the amount of leaf rust was less than it had been at an earlier stage, while the stem rust was at a maximum. At maturity the tops were cut at the soil level, dried for two days at  $105^\circ \text{C.}$ , and weighed. The grain was then hand threshed, dried again, and weighed. The data for the various cultures in each series are presented in Tables II and III. A direct comparison is thus permitted of a set of plants free from rust with a set each of leaf-rusted and stem-rusted plants which grew under identical conditions. Since there were only four plants in

each culture, great reliance can not be placed on these results; yet, taking into account the extent of agreement within each culture indicated by the computation of the probable error, certain significant differences appear to result from some of the treatments.

TABLE II.—Yield and water requirement of Marquis wheat plants grown in each of ten cultures, consisting of four replicate crocks, in rusted and rust-free series

Culture.	Yield.		Water used.	Water requirement.		Per cent rust.	
	Tops.	Grain.		Tops.	Grain.		
	Gm.	Gm.	Cc.				
OO:							
Stem rust	19.484±0.893	2.304±0.230	6,524±307	336±5.0	3,079±389	25±6.9	
Leaf rust	20.751±.812	2.638±.527	6,822±308	328±5.9	2,676±254		8±1.7
Control	19.219±.763	3.320±.220	6,625±208	346±6.8	2,044±131		Tr.
OA:							
Stem rust	16.817±1.4	1.410±.238	6,226±247	380±20.0	7,645±275	35±13	4
Leaf rust	18.884±.067	1.797±.236	6,451±244	342±3.5	4,300±192		8±.94
Control	17.003±1.82	1.911±.560	6,348±163	327±8.6	3,518±53		
OB:							
Stem rust	18.123±.36	1.717±.283	6,865±169	379±1.7	5,630±1,646	40±11	
Leaf rust	21.550±1.26	1.862±.345	6,167±163	293±17.5	4,735±1,019		9±1.3
Control	20.972±.491	2.453±.246	6,700±43	324±5.1	2,964±369	Tr.	Tr.
IA:							
Stem rust	21.235±.378	1.670±.337	7,552±159	357±12.0	6,146±2,713	65±5.4	
Leaf rust	24.496±.307	2.355±.503	7,145±219	291±7.2	3,094±154		18±1.3
Control	23.711±.797	2.868±.323	6,625±129	259±13.0	2,948±266		Tr.
IB:							
Stem rust	21.138±.122	1.263±.339	6,785±71	327±15.0	9,749±2,120	30±4.9	
Leaf rust	23.953±.423	1.744±.288	6,633±161	277±3.9	4,971±1,178	Tr.	32±1.6
Control	25.824±.433	1.123±.258	7,022±164	272±2.9	8,143±1,275		Tr.
IIA:							
Stem rust	19.563±1.55	1.931±.215	6,358±294	333±16.0	4,357±987	25±1.6	
Control	23.430±.507	1.375±.242	6,919±117	309±3.5	6,252±2,169	Tr.	Tr.
IIIB:							
Stem rust	16.924±.31	0.893±.46	6,109±8	362±7.6	7,973±180	45±6.8	
Control	19.677±.332	1.256±.396	6,443±20.4	328±9.1	5,518±624		1
IIIA:							
Stem rust	19.307±.824	1.598±.534	6,189±291	320±5.3	4,159±410	3±.67	
Leaf rust	18.379±.888	1.394±.266	5,983±88	327±6.8	5,598±976	Tr.	2±.06
Control	21.635±.671	1.738±.392	6,646±150	307±2.0	4,259±874		Tr.
IV:							
Stem rust	20.122±.535	3.356±.315	6,016±215	299±.18	1,937±257	15±2.9	
Leaf rust	19.121±.526	4.069±.390	5,445±242	285±10.0	1,406±111		4±.67
Control	23.828±.373	5.605±.212	6,808±116	286±4.2	1,223±31	Tr.	1
VB:							
Stem rust	20.562±1.72	2.236±.442	5,816±111	293±5.7	2,655±756	8±1.3	6±.94
Leaf rust	14.288±1.32	0.986±.438	4,588±337	327±14.0	5,293±795		1
Control	22.155±.573	1.885±.402	6,657±178	301±4.5	3,619±210		

<sup>a</sup> Indicates stem-rust infection.

<sup>b</sup> Indicates leaf-rust infection.

TABLE III.—Influence of stem rust and leaf rust on yield and water requirement of Marquis wheat, based on average results from ten different nutrient cultures, each consisting of four crocks <sup>a</sup>

Culture.	Dry weight, mean.	Water used.	Water requirement.	Per cent rust.
	Gm.		Cc. per gm.	
Stem rust:				
Tops	19.327±0.345	6,444±109	339±6.5	30±5
Grain	1.838±.139		5,333±591	
Control:				
Tops	21.652±.526	6,679±41	306±5.8	.5±.2
Grain	2.353±.272		4,004±459	
Leaf rust:				
Tops	20.178±.801	6,154±106	308±8.7	10±1
Grain	2.015±.219		4,009±386	

<sup>a</sup> Data from the immature plants, including culture IIIB in all series, and the plants in culture IIA and IIB of the leaf rust series are omitted.

## DISCUSSION

An examination of Table III shows less difference in yield of tops and grain, and of water requirement based respectively on these, than might be expected. It is to be borne in mind, however, that the reduction in yield is not in this case complicated by diminution of the soil moisture to a dangerous point, as frequently occurs in the field when drought and rust are coincident. Furthermore, the prolonged growth period undoubtedly resulted in an abnormally high water requirement for all cultures, whether rust-infected or not, and tended to eliminate more marked differences that might have been evident in a shorter period of rapid growth.

In connection with their extensive researches on the water requirement of crop plants, Briggs and Shantz (3, 4, 5) summarized the results reported by earlier investigators. Thus the water requirement for wheat (tops), as reported in literature, ranged from 235 found by Lawes at Rothamstead to 554 found by Leather in India. The results here reported lie well within this range. Briggs and Shantz found the average water requirement for six varieties of wheat to vary from 400 to 500 in different years. The water requirement based on grain production, in the present instance, is abnormally high in nearly every case, being comparable to that reported originally by Briggs and Shantz for plants growing under conditions unfavorable to economical use of water, and more than twice the figures given in their later papers (*loc. cit.*). However, at least one culture, IVB of the control series, compares favorably with their results in all respects. The following conclusions seem to be warranted by the data:

Infection by either stem rust or leaf rust results in a significant reduction of yield of tops and of grain that is independent of the soil moisture supply, and this reduction is evident with only moderately heavy infection. Taking the figures for the stem rust and control series at face value, it appears that an amount of rust estimated at 30 per cent may cause a 20 per cent reduction in yield even though the soil moisture content remains at a favorable level.

Accompanying the reduction in yield, there is practically as great use of water as in healthy plants; that is, rusted plants have a higher water requirement, based on yield of both tops and grain. This is shown to be significant only in the case of stem rust and is of a higher order for grain than for total dry matter. The lesser effect on yield of tops, resulting from rust infection, is probably due to the fact that the soil water supply was at all times sufficient; under field conditions dry weather may greatly diminish the yield of the entire plant.

The effect of rust in diminishing yield and increasing the water requirement seems less likely to be due to increased transpiration, resulting from rupture of the epidermis at postule formation than to other causes, for the total amount of water used by rusted and healthy plants is about the same. Leaf rust, which produces but slightly erumpent pustules as compared with stem rust, and hence less injury to the epidermis, also depresses yield, more particularly that of grain, but also of tops, though in each case to a less extent. Chief among the causes of yield reduction arising from rust infection are to be reckoned the destruction of chloroplasts in the infected tissue and the drain on the elaborated food reserves of the host.



## EFFECT OF CERTAIN MINERAL NUTRIENTS ON DEVELOPMENT OF RUST AND INJURY TO HOST

The original plan of this investigation included an extensive study of the effect of mineral nutrition upon the host as regards its susceptibility to rust infection and the injury it suffers therefrom. Owing to the fact that it was not possible to repeat the experiment described in Part I of this paper, or to extend the investigation along different lines, it seems best to draw no conclusions in respect to these questions from the limited data at hand. Certain tendencies were noticeable, however, as regards the performance of the plants under some of the treatments, and the data from all cultures which attained approximate maturity are presented in Table IV, arranged to show the effect of the mineral nutrients on the yield and quantity of rust.

TABLE IV.—*Influence of different culture media on average yield and water requirement of two cultures of Marquis wheat, one rusted and one rust-free, each consisting of four replicate crops*

Culture.	Yield.		Water used.	Water requirement.		Average per centage of rust for two infected cultures.
	Tops.	Grain.		Tops.	Grain.	
	Gm.	Gm.	Cc.			
OO.....	19.818±0.822	2.754±0.326	6,657±274	336± 5.9	2,600± 258	17
OA.....	17.588±1.095	1.706± .361	6,342±218	350±107.0	5,154± 173	24
OB.....	20.215± .703	2.011± .291	6,579±125	332± 8.1	4,443±1,043	25
IA.....	23.147± .494	2.298± .387	7,107±169	302± 10.4	3,913±1,044	42
IB.....	23.638± .326	1.377± .261	6,813±132	292± 7.2	7,623±3,567	31
IIA <sup>a</sup> .....	20.997±1.028	1.653± .228	6,639±205	321± 9.7	5,305±1,578	13
IIB <sup>a</sup> .....	18.300± .321	1.075± .428	6,276±106	345± 8.3	6,746± 402	23
IIIA <sup>b</sup> ....	19.612± .794	1.335± .397	6,267±176	320± 4.7	7,765± 753	3
IVB.....	21.024± .478	4.343± .305	6,090±191	290±104.0	1,522± 136	10
VB.....	19.002±1.204	1.702± .427	5,687±208	307± 8.0	3,856± 587	8

<sup>a</sup> Data for leaf rust series omitted owing to immaturity of the plants.

<sup>b</sup> Series IIIB, with excess KCl, omitted owing to immaturity.

Even a greatly curtailed review of the literature of transpiration dealing with the effects of nutrient ions and other substances and the state of fertilization upon the water relations of plants, will not be attempted here. Briggs and Shantz (3, 4) have given a very thorough review of the literature dealing with water requirement based on mature growth of plants, and a bibliography of papers containing water requirement measurements based on seedlings. A large number of papers dealing only with rate of transpiration as affected by various substances, of nutritive value and otherwise, is included in the comprehensive review of the subject of transpiration by Burgerstein (6). The literature dealing with the effect of fertilization on the severity of rust is reviewed by Raines (12).

Briggs and Shantz conclude that a reduction in the water requirement results from the use of fertilizers, although very little difference in this respect is shown by highly productive soils. A highwater requirement may result from deficiency of a single nutrient element, or in fact from any condition which operates to limit or retard growth, as measured by carbon assimilation, while transpiration suffers no corresponding change. Furthermore, a highwater requirement may be

brought about by a soil moisture content which tends to be too low or too high for unrestricted growth, or, in the case of water cultures, by great dilution of the solution.

An examination of Tables II and IV shows, first as regards the yield of tops and grain obtained from the various nutrient treatments, that only cultures OO, OB, IA, and IVB have produced yields comparable to ordinary yields of field-grown plants—i. e., above 15 bushels per acre. Culture OO is similar to Shive's  $R_3C_3$ , which was found most suitable for growth of wheat seedlings during the first 30 days. It is to be noted that in yield of grain this culture was exceeded only by IVB containing  $CaCl_2$ . Even the addition of nitrate, an ion which Hoagland (10) has shown may be absorbed very rapidly from solutions and which may therefore be deficient, particularly during the period of greatest absorption, in sand or solution cultures carried on as these were, resulted only in increasing the yield of tops. The reason for the striking increase in grain produced by the addition of  $CaCl_2$  to the Shive solution is not clear. It can hardly be attributed to the direct nutritive value of calcium, but is more likely to be accounted for either by an improvement in the reaction of the culture medium or by rendering other ions "physiologically available" (True, (21)). A depressing effect on plant growth resulting from too high acidity of the medium has been observed by a number of recent investigators, and it has been shown that a correlation exists between acidity of the cell sap and retarded growth (20). Whatever the explanation, it is significant in this case that the Shive solution, modified by the addition of 0.0171 gram molecules of  $CaCl_2$  per liter, has produced a growth of mature wheat that in respect to yield and water requirement is comparable to that of plants grown under the best pot culture methods that have been devised or with that of field-grown wheat. The low-water requirements shown by these plants, both for tops and for grain, is in line with expectations if one of the functions of calcium, as stated by True (21), is to maintain normal permeability relations of the cell wall.

As regards the effect of mineral nutrition on the development of rust, it appears that the application of an excess of nitrate has resulted in greater susceptibility to rust in both the leaf-rust and stem-rust series. In Culture IA, where the additional nitrogen did not so greatly delay maturity, the infected plants show a much reduced yield of grain also. The appearance of the plants themselves, particularly in the leaf-rust set, confirmed their more ready susceptibility, as both the number and size of pustules were greater on plants receiving additional nitrogen. The more luxuriant development of culms and the more rapid growth of these plants seem sufficient to account for the greater development of rust, and until further evidence is at hand, the assumption of a physiologically greater susceptibility to rust in nitrogen-fertilized plants is unnecessary.

The slight development of rust on the plants receiving calcium and magnesium is noteworthy since in these cultures the effect can not be attributed to generally poor growth, which is the case in those fertilized with phosphate and potash. It appears that the plants of Cultures IV and V actually were somewhat physiologically resistant, since repeated attempts to infect them were only partially successful. Hurd<sup>6</sup> indicates

<sup>6</sup> HURD, Annie M., THE ACIDITY OF SOME RUST AND SMUT RESISTANT AND SUSCEPTIBLE VARIETIES OF WHEAT AND SOME FACTORS AFFECTING IT. Abstract presented at the meetings of the Physiological Section, American Bot. Soc., at Toronto, Canada, Dec. 29, 1921. (Mimeographed.)

a correlation between the total acidity of the cell sap of certain varieties of wheat and resistance to rust and smut; also that the total acidity (content of buffer substances) may be decreased by liming the soil. Should a similar correlation have existed in the plants constituting Culture IV of the present experiment, their "resistance" to rust must have been due to something other than the reaction of the sap.

### SUMMARY

Marquis wheat was grown to maturity in quartz sand cultures supplied with various combinations of mineral nutrients added in solution. An artificial epiphytotic of leaf rust, *Puccinia triticina*, was induced in one series, and of stem rust, *P. graminis tritici*, in a second, while a third was maintained free from infection.

Rust infection of either type resulted in lowered water economy of the host, whether the dry matter of entire tops or of grain is considered. The actual quantity of water transpired is of significance in relation to infection only when the correlative production of dry matter is taken into account.

The addition of NaCl or  $\text{NaH}_2\text{PO}_4$  to the basic three-salt nutrient solution did not affect the susceptibility of wheat to leaf rust or stem rust. The addition of  $\text{NaNO}_3$  resulted in somewhat readier infection in each case but did not predispose to greater injury. KCl retarded infection in proportion to the diminution of growth of the host, particularly when used in excess.  $\text{CaCl}_2$  and  $\text{MgCl}_2$  appeared to induce a state in which the host was less readily susceptible to infection.  $\text{CaCl}_2$  also resulted in a reduction of water requirement, about 10 per cent for tops and 40 per cent for grain.

The addition of 0.0085 and 0.0171 gram molecule of  $\text{NaNO}_3$  per liter and 0.0171 gram molecule of  $\text{CaCl}_2$  caused an increase in yield over that obtained from the Shive  $\text{R}_3\text{C}_2$  solution as here used.

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## PHOTOPERIODISM IN RELATION TO HYDROGEN-ION CONCENTRATION OF THE CELL SAP AND THE CARBOHYDRATE CONTENT OF THE PLANT<sup>1</sup>

By W. W. GARNER, *Physiologist in Charge*, C. W. BACON, *Physiologist*, and H. A. ALLARD, *Physiologist, Tobacco and Plant Nutrition Investigations, Bureau of Plant Industry, United States Department of Agriculture*

### INTRODUCTION

In previous papers (7, 8)<sup>2</sup> it has been shown that in many species the duration of the daily period of illumination exercises a remarkable formative action on plant development, and in particular may initiate or inhibit flowering and fruiting. The great majority of species, perhaps all of them, are more or less responsive to differences in the daily light period, though species and varieties differ widely in their sensibility to this influence. In many species the action of the light period under ordinary circumstances is dominant, so that the response of the plant is prompt and certain under a rather wide range of conditions as to temperature, water supply, intensity and composition of the light and the supply of plant nutrients. For example, spring plantings of the four varieties of soy beans (*Soja max* (L) Piper) known as Mandarin, Peking, Tokyo, and Biloxi, regularly begin flowering in the latitude of Washington about June 15, July 10, August 1, and September 1, respectively, and it has been demonstrated that these differences in time of flowering are characteristic varietal responses to the seasonal change in length of day. Under normal conditions the later varieties are unable to flower until appropriate decrease in length of day has taken place. By artificial shortening of the daily light period all varieties are made to flower at approximately the same time, while, on the other hand, the later varieties may be maintained in the active vegetative condition for a prolonged period of time by continued exposure to a relatively long daily period of illumination (in excess of 13 hours). Artificial light may be effectively used as a supplement to sunlight in prolonging the daily light period. Many other species have been found to be equally responsive to change in the light period, so that in such cases flowering and fruiting may be induced or suppressed at will by proper regulation of the daily illumination period.

Plants which behave like the later varieties of soy beans, that is, tend toward indeterminate increase in stature without flowering when exposed to a relatively long daily light period but quickly flower in response to suitable decrease in the light period, are for convenience designated as short-day plants. There is another large group of plants which tend to remain in the leaf-rosette stage, without stem elongation, and fre-

<sup>1</sup> Received for publication Sept. 12, 1923.

<sup>2</sup> Reference is made by number (italic) to "literature cited," p. 155-156.

quently with some form of tuberization when exposed to a short light period, but are capable of developing flowering stems under the influence of a longer daily light period or under certain conditions even when exposed to continuous illumination. Plants of this type are designated as long-day plants.

In both of the above-named groups the rate of growth of the stem increases with increase in the length of the illumination period, maximum growth being associated with sterility in the first group and with flowering and fruiting in the second group. There is a third group, however, in which maximum stem growth is attained with a light period of intermediate length approximating the equatorial length of day. In this group a light period in excess of the optimal for growth favors flowering and fruiting and tuberization. In all cases observed the height of the flower stem, like that of the vegetative stem, increases with increase in the length of the light period, provided the limits favorable to flowering are not exceeded. The bearing of these group relationships on the natural distribution of plants and on the behavior of a given species in different latitudes will be apparent at once to the ecologist. The duration of the light period may induce or modify various other forms of response such as branching habits, root growth, leaf fall and dormancy, but the present discussion will be limited primarily to the fundamental relationships of increase in stature, sexual reproduction and tuberization.

One of the most striking features of the response to duration of the light period so far as concerns initiation of flowering is that the important factor is not merely the total number of hours of illumination during the 24-hour period but the number of hours of uninterrupted light in each period of exposure. Thus, darkening soy beans and other species during the middle of the day for periods of four, five, or six hours, beginning at 10 a. m., has but little effect in either hastening or delaying the advent of flowering, though the growth rate may be materially checked.

Another significant fact is that in many cases electric light of intensity as low as 5 foot-candles when used for prolonging the daylight period is capable of exercising a definite formative action in either initiating or inhibiting flowering. It is difficult to explain the action of light of such low intensity on the basis of photosynthesis alone. While the effect on sexual reproduction is clearly expressed, the general nutrition of the plant is not permanently maintained and sooner or later decline sets in, usually resulting in death.

Since sexual reproduction and other forms of expression can be readily controlled by proper regulation of the daily light period this factor furnishes a means for making detailed study of the internal conditions of the plant which are associated with alternative forms of development, at least in so far as analytical methods are available for such work. Under proper conditions and with suitable material the time required for definite expression is easily determined and varies only within narrow limits. Moreover, it is not necessary to control accurately other environmental factors in order to obtain the expected response. It is believed that a large field for profitable study of internal environment in relation to response is thus opened up and the present paper is intended as a beginning in this field. But little is available in the way of a scientific background for such studies, for thus far the duration of the light period as a factor in plant development has received almost no attention from physiologists and biochemists. Even in the case of photosynthesis, while there is a voluminous literature on the effect of intensity and

composition of light, there are no satisfactory data to show whether in any particular species the process proceeds at the same rate and in the same manner with a daily illumination period of, say, 15 hours as with a period of 10 hours. The same is true of transpiration, respiration, and various other plant processes of fundamental importance. The present paper deals with fairly extensive studies of hydrogen-ion concentration of tissue fluids and some preliminary data on carbohydrate content and water relations as affected by the length of the daily light period, and thus associated with alternative forms of expression.

#### WATER RELATIONS IN PHOTOPERIODISM

Thus far only a beginning has been made in direct experimental study of the influence of the light period on water relations in the plant, but in connection with the other studies discussed in this paper it seems desirable to direct attention to observed plant reactions which suggest a definite relationship between the length of the illumination period and the water content of the plant. That maintenance of maximum turgidity is in some way favored by a light period which is optimal for increase in stature is illustrated by the fact that in certain cases transfer from such light conditions to those which are suboptimum for increase in stature quickly results in a change from the upright toward the horizontal or prostrate position of the stem, although growth may be maintained. In other cases, this change in the light relations causes dying back of the stem, followed by development of new basal shoots. Moreover, increased pubescence may be a feature in the change from an optimal to a suboptimal light period for upward stem growth. These phenomena and other features of development commonly seen under relatively xerophytic conditions are observed when the plants are abundantly supplied with water and even under fairly wide ranges in the external water supply.

It is well known that as a rule exposure to sunlight greatly increases transpiration and it seems possible that in those species for which an intermediate length of day is optimal for growth the check in rate of growth resulting from exposure to long days is due at least in part to excessive transpiration. For other species, however, the rate of increase in stature is more or less proportional to the length of the daylight period and loss of turgidity resulting from decrease in the daily light period would seem to be due to changes in internal conditions of the plant. From the investigations of Briggs and Shantz (4, 5) and Livingston (14), together with the work of earlier investigators, it might be assumed that in the first case the excessive transpiration (if such actually occurs) is probably due chiefly to external evaporative forces. In those species in which maximum turgidity is maintained by exposure to a long daily illumination period internal factors are apparently involved, for it is not clear as to how reduction in the period of illumination could through direct action cause decrease in turgidity as a result of excessive transpiration. According to Livingston and Brown (15), exposure to sunlight may result in incipient drying of the foliage leaves, which in turn may tend to check transpiration to some extent by causing partial closure of the stomata and decreased partial pressure of water vapor in the leaf. There seems to be no ground at present, however, for supposing that increased duration of the light period would tend to accentuate the effectiveness of these factors in checking transpiration to the point of overcoming external evaporative forces. Changes in osmotic relations,

permeability of the protoplasmic layer and the hydration capacity of the hydrophilic colloids, might be regarded as possible factors in the reduced water-holding capacity of the plant tissues when the plant is exposed to illumination periods which are too short for maximum rate of increase in stature.

Prompt initiation of sexual reproduction when certain species are exposed to a reduced daily illumination period is perhaps the most striking phenomenon of photoperiodism, and in the case of the violet, at least, this response has been shown to be associated with partial loss of turgor. Here again is seen a possible relationship with the well-known fact that flowering and fruiting are favored by conditions of comparative drought, as has been especially emphasized by Möbius (20). That darkening species of this type for several hours in the middle of the day fails to initiate flowering possibly may be due in part to resultant check in rate of transpiration, thus enabling the plant to maintain higher turgidity. There is the possibility that the reduced rate of growth under these conditions is explainable on the basis of reduced photosynthesis.

There can be no doubt that the water relations of the plant are profoundly affected by the duration of the daily illumination period, but it is impossible to state whether change in water content stands in a direct causal relation to observed responses of the plant or merely follows as a sequel to other internal processes more directly controlled by the light period.

#### ACIDITY RELATIONS IN PHOTOPERIODISM

The theory advanced by Liebig (13) that organic acids are intermediate products in the photosynthesis of carbohydrate has not met with favor among plant physiologists and it has come to be the generally accepted view that these acids result from partial oxidation of carbohydrate and fat. While formation of organic acids thus seems to be more directly related to respiratory activity than to photosynthesis, there is no doubt of the fact that light is in some way a dominant factor in the origin of these catabolic products, as will be developed more fully in the present discussion. Some of the acids formed are neutralized by mineral bases derived from the soil, and to the extent that insoluble salts are formed these tend to accumulate in the plant tissues. According to de Vries (25), however, the total quantity of acids formed in a period of 100 days may greatly exceed the total dry weight of the leaf, so that obviously the greater portion of the acids formed must undergo decomposition, the final products being carbon dioxide and water. It is generally believed that light is a very important factor in the breaking down of organic acids in the plant cell. It is apparent that the total acidity at any particular moment is dependent, on the one hand, on the rate of formation of acids and, on the other hand, on the rate of their decomposition and, to some extent, perhaps, on the quantity neutralized by bases derived from the soil. In addition to light, temperature has been found to be one of the important external factors influencing relative rate of formation and decomposition of acid, high temperatures favoring net decrease in acidity.

With respect to comparative total acidity of different organs of the plant and of particular organs at different stages of development the most extensive previous observations are those of Astruc (1) who investigated the total free acid content of a number of species. This inves-



tigator, however, failed to recognize the effect of alternative forms of activity on the distribution of acidity in the different parts of the plant, so that his results as a whole can not be readily interpreted. In brief, he found that in the leaf the acidity decreases with increasing age of the organ, in the stem the acidity decreases from the apex downward, while in the floral organs acidity decreases from the button stage up to unfolding of the blossom, and the developing fruit also shows decreased acidity.

Various functions in the economy of the plant have been ascribed to organic acids, but detailed discussion of the different theories which have been advanced on the subject will not be here undertaken. It was long ago suggested that the free acids and their soluble salts play an important rôle in promoting osmotic pressure, turgor, and growth, and this theory in its relation to change in the acidity of plants when exposed alternately to light and to darkness has been widely discussed (cf. Kraus (12)). This is the most important of the older theories regarding the effect of acid content of the plant fluids on metabolism, and the general procedure which has been followed in studying the subject involves measurement of total acidity by titration. In the application of more recent conceptions regarding the effect of acidity on the properties of plant colloids, however, it becomes necessary to give special consideration to relations of active acidity<sup>3</sup> of the tissue fluids, as measured by concentration of hydrogen ions. The latter phase of plant acidity has not thus far been fully developed. According to Atkins (2) plant cells rarely show an alkaline reaction and the  $P_H$  value of the sap does not exceed the number 8. On the other hand, in certain fruits the  $P_H$  value of the sap may fall as low as 1.4. According to this author "the  $P_H$  value met with in a tissue is usually near, but slightly less than, the optimum for the activity of the characteristic enzyme at ordinary temperature." Clevenger (6) studied the range in hydrogen-ion concentration in leaf, stem and root of cowpeas during the 24-hour period, and found the highest concentration in leaf and stem to occur during the morning hours, while the lowest concentration was observed during the night. The root showed a narrower daily range in acidity and the maximum occurred during the day. Of the three plant parts the stem averaged highest and the root lowest in active acidity. From observations on several crop plants Haas (10) finds that the active acidity of the sap is affected by changes in illumination, the soil solution, the age of the plant, and other conditions. Several publications have appeared in recent years which deal with the hydrogen-ion concentration of the cell sap as affected by the reaction of the soil solution or the culture medium, but these problems are not directly involved in the present discussion and hence may be passed over.

The significance of measuring hydrogen-ion concentration in studying the relation of acidity to the properties of colloidal proteins has been especially emphasized by Loeb (16). In view of the effects of acidity, as thus measured, on the swelling of gels and on the viscosity and osmotic pressure of protein solutions, it is desirable to obtain much more complete information than has hitherto been available as to the influence of external environment on the hydrogen-ion concentration of the cell fluids. It is hoped that the present contribution will add materially to

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<sup>3</sup>The term "active acidity" refers to the portion of any acid substance which has become ionized and thereby rendered active in producing the effects commonly ascribed to acids; it is measured in terms of hydrogen-ion concentration.

the available data on the effect of light, and more specifically the duration of the daily illumination period, on the active acidity of the cell sap.

This problem is complicated by the marked differences in acidity which are found in the different organs and tissues of the plant under varying environmental conditions. An additional complication is the progressive change in acidity relations with increase in age of the individual organs and of the plant as a whole. In this connection, it is necessary also to keep in mind the fact that the "age" of the organ or organism, as indicated by the stage of development attained, can not be measured solely by lapse of time but is dependent largely on the particular environment to which the plant happens to be exposed. To give a concrete illustration, when late-flowering cosmos is exposed to a daily illumination period of, say, 15 hours, the apex of the stem shows a progressive and marked increase in hydrogen-ion concentration as growth proceeds, maximum acidity being attained only after the lapse of several weeks. In this case vegetative development of the stem continues for a more or less indefinite period. On the other hand, if exposed to 10 hours of light daily, the apex of the stem shows no such marked increase in active acidity until after the flower bud has appeared. Under the longer illumination period the active acidity of the apex is much greater than that of the base of the stem, while under the shorter illumination period these relations may be actually reversed. Proper sampling of the material for study offers considerable difficulty, since for practical purposes it is often impossible to separate completely tissues, or organs, even, especially in the earlier stages of development. In most cases, however, the differences in acidity are so well defined as to leave no doubt as to the adequacy of the methods of sampling followed, where only comparative results are required.

In these experiments, except in special cases, no effort was made to follow out in detail the change in acidity through the 24 hours of the day, for such a procedure is out of the question where it is necessary to conduct a large number of separate tests. This difficulty was overcome as far as possible by choosing different hours of the day for making the observations in the course of a particular series, always collecting samples at as nearly the same hour as possible in comparing material exposed to two different illumination periods. In certain cases, moreover, comparative readings were made in the morning and afternoon hours, as a further check on the results obtained. In all cases the hour at which sampling was done was recorded and the material was used for observation as soon as possible after it had been collected.

In carrying out the measurements the plant material was crushed in a porcelain mortar or in a tinned food chopper and the crushed material was strained through several thicknesses of cheesecloth. The hydrogen-ion concentration was determined electrometrically, the potential being measured with either a Northrup "millivoltmeter" or a Northrup hydrogen-ion "pyrovoltmeter" and a Leeds and Northrup small, portable galvanometer. The saturated KCl-Calomel electrode was used and the hydrogen electrode was made and "platinized" essentially as directed by Bovie (3). The platinum coat on the wire, however, was always deposited just before being used. Electrolytic hydrogen compressed in cylinders was caused to bubble through the sample of sap and the potential was first observed after a period of 10 minutes. Further observations were made at intervals of 5 to 10 minutes until a constant reading was obtained,

this usually requiring 10 to 20 minutes. The temperature of the air was noted and the  $P_H$  value<sup>4</sup> of the sample calculated. The accuracy of the outfit used was frequently checked by observing the potential of a standard acetate solution or of a solution of potassium acid phthalate.

#### DAILY PERIODICITY IN ACIDITY OF THE CELL SAP

Before passing to detailed consideration of the effect of differences in the light period on the average level of plant acidity it is necessary to discuss briefly the subject of the daily change in acidity during the 24-hour period, involving both the formation and the decomposition of acid. That there is a rhythmic daily change in acidity in succulents has been known since [1813], when Heyne (11) made the observation that leaves of *Bryophyllum calycinum* possess a more acid taste in the early morning than in the afternoon. Quantitative observations on the subject were made by A. E. Mayer (17) in 1875, and he confirmed the fact that in succulents there is an increase in acidity at night and a decrease during the day. Similar periodicity, however, could not be detected in *Oxalis* species. Mayer found that in the absence of  $CO_2$  *Oxalis* gives off no oxygen when exposed to sunlight, and therefore concluded that organic acids are not intermediate products of photosynthesis. *Bryophyllum*, on the other hand, does evolve oxygen gas in sunlight in the absence of an external supply of  $CO_2$ . Increase in temperature from 20° to 30° C. causes a decrease in acid content in *Oxalis*, which was ascribed to increased respiratory activity. In later publications (18, 19,) Mayer undertakes to show that the evolution of oxygen by succulents when exposed to sunlight in an atmosphere free from  $CO_2$  is due to splitting of oxygen from the  $CO_2$  which is formed by the plant and again utilized in photosynthesis. G. Kraus (12) studied various phases of acidity relations in plants. He observed a decrease in acidity in several species of the nonsucculent type when exposed to sunlight and, as a result, made the mistake of assuming that the phenomenon of increase in acidity at night and decrease during the day applies quite generally to nonsucculents as well as to succulents. Kraus found that deacidification is due to direct action of light and is not dependent on either respiration or photosynthesis. This investigator concluded that the content of mineral salts of organic acids in the tissues does not change materially from day to night or from day to day, hence daily periodicity in acidity is not due to the neutralizing action of bases derived from the soil.

In an important contribution to the subject of plant acidity de Vries (25) brought out several facts of special significance in their bearing on the present discussion. The marked increase in acidity in succulents occurring at night is due to previous action of sunlight, for the nocturnal rise in acidity does not occur in the absence of illumination by day. Moreover, in continued darkness there is progressive decrease in acidity during both day and night after the first 24 hours, and this phenomenon seems to apply to thin-leaved species as well as to fleshy plants. The action of sunlight in causing subsequent rise in acidity is not due to a heat effect, for warming in darkness during the day does not cause increased acidity at night. Also, the action of light is not due directly or solely to photosynthesis, for exposure to light in an atmosphere free from carbon dioxide

<sup>4</sup> It may be pointed out here that the  $P_H$  value represents the potential due to hydrogen ion in a solution, and is the negative logarithm of the actual concentration of this ion. Hence, the higher the acidity, the smaller the  $P_H$  number; and a change of one  $P_H$  unit means a tenfold change in hydrogen-ion concentration.

causes rise in acidity at night. Very weak light which could not cause marked photosynthetic activity is able to promote nightly rise in acidity. A very short period of illumination, on the other hand, causes no rise in acidity at night. The increase in acidity at night is due to a greater rate of formation than of the destruction of acid, while under high temperatures the rate of destruction may keep pace with the rate of formation. De Vries concludes that accumulation of acids at night is largely limited to fleshy plants. Finally, light action is not the cause of deacidification, but merely promotes the process. Warburg (26) in his paper on the subject gives a thorough review of previous work, and records observations on a large number of species. This author finds that decrease in acidity in the presence of sunlight does not take place in all thin-leaved species. In general, the daily decrease in acidity is proportional to the degree of protection against transpiration afforded by the structure of the plant organ. Plant parts which are free from chlorophyll show little or no decrease of acidity in sunlight. In the green parts of succulents deacidification both in the presence or the absence of light is coupled with the presence of oxygen. Increase in acidity at night is probably dependent on increase in sugar content which results from illumination during the day. The presence of oxygen, though in only small quantity, is required for acid formation. The acids of succulents are to be regarded simply as products of incomplete oxidation. The formation of acids in plant tissues is proportional to (1) the intensity of metabolism, (2) the degree of protection against entrance of oxygen; the decomposition of acids is proportional to (1) the intensity of metabolism, (2) the accessibility of atmospheric oxygen, (3) the temperature.

Purievich (22) finds that decrease in acidity of leaves in prolonged darkness is not due to physiological translocation or to neutralization of the acid by bases derived from the soil. The formation of acid in the dark is proportional to the intensity and duration of the preceding illumination and leaves placed in sugar solutions showed increased formation of acid. Transfer from the light to darkness causes a gain in acidity for a period ranging from 8 hours in some species to more than 24 hours in other species, and these differences are believed to be correlated with differences in the relative stability of malic, oxalic, tartaric, and citric acids, since different species do not contain the same acids. In the spontaneous decomposition of solutions of these acids *in vitro* when exposed to sunlight malic acid is most easily broken down, followed by tartaric and oxalic, respectively, while citric acid is not affected. Oxalic and malic acids, however, are most affected by increase in temperature. In a comparatively recent review and study of acidity in succulents, with special reference to cacti, Richards (23) finds maximum acidity of the plant juice at about 7 o'clock a. m. and the minimum at about 5 p. m., in the case of *Opuntia versicolor*. The greatest daily range in acidity occurs in summer. The daily range was greater in younger joints of the plant than in older ones. The diurnal change in acidity is due mainly to sunlight, while temperature also is a factor. The formation of acid in the plant is due to an inadequate supply of oxygen in the tissues. Deacidification is not believed to be a part of the respiratory process. As bearing on the work of Purievich relating to the relative rate of decomposition of different acids in sunlight and the more recent study of the subject by Spoehr (24), attention is called to the fact that the same difference in ease of decomposition between malic and citric acids applies to deacidification in darkness, as has been

shown in this office in the case of tobacco leaves during the process of curing (9). During the progress of the curing there is a decided decrease in total content of malic acid, while there is an equally marked increase in content of citric acid.

From the above-mentioned investigations it appears that clearly defined daily periodicity in acid content, with marked decrease in acidity during the daylight period and a corresponding increase in acidity at night, is characteristic of succulents having structural features which are unfavorable for rapid gas exchange. This periodicity is much less evident in thin-leaved species and in some of these has not been observed to occur at all. Formation of acids in plant tissues results from incomplete oxidation of carbohydrate and is favored by a limited oxygen supply. While, in the case of succulents, the acids accumulate chiefly during the night, nevertheless the rate of acid formation is in some way dependent primarily on the conditions of illumination during the day. Decomposition of acids in the tissues of the plant is facilitated by increased oxygen supply, by exposure to light, and by relatively high temperatures. Exposure to light, however, is not a necessary condition for acid decomposition. There seems to be considerable doubt, moreover, as to whether deacidification is to be regarded as a part of the respiratory process.

The present investigations, relating primarily to the average level of acidity of the plant sap as affected by the duration of the light period, have incidentally emphasized the relatively narrow daily range in acidity of the thin-leaved species which have been studied. Direct observations also have been made on this point, in the case of Biloxi soy beans. A series of readings were obtained on the sap of the young, topmost leaves of the plants collected early in the morning, at noon, and late in the afternoon. In plantings made in the field the average  $P_H$  values of the saps during the midsummer, when the plants were most active vegetatively, were 6.22, 6.19 and 6.14, respectively, for the samples collected in the morning, at midday and in the afternoon. Similar readings taken later in the season, when the plants were approaching or had actually reached the flowering stage, showed practically no change in active acidity during the day. These results were confirmed by a second series of observations on plantings made in boxes and exposed to the natural length of day of early and late summer. Finally, in plantings in boxes exposed to only 10 hours of light daily, a light period which, of course, quickly initiates flowering, there was at most only a slight increase in acidity during the day. Thus, in contrast with the behavior of succulents, Biloxi soy beans show an appreciable increase in hydrogen-ion concentration of the sap from morning to afternoon as long as the plants continue in the active vegetative stage as a result of exposure to relatively long days. When the flowering stage is reached as a result of exposure to relatively short days there is little or no daily change in active acidity.

In plants which show the behavior of succulents it might be expected, perhaps, that an increase in the number of hours of daily illumination would tend to reduce the average acidity for the 24-hour period. In any event, with respect to thin-leaved species it may be stated that in the limited number observed in the present investigation the general tendency has been toward an increase in average active acidity with increase in duration of the daily light period. This holds true in general both

for plants which flower when exposed to relatively short days and for those which flower when exposed to long days. In detail, however, there are important differences in the actual course of the acidity level during growth and development in the two classes of plants, for changes in form of development resulting from differences in the light period are associated with definite changes in average acidity of the cell sap. Moreover, decrease in the duration of the light period to which the plant is exposed may not result in immediate decrease in average active acidity. Apparently, the full effect of such change in illumination is not seen, as a rule, till about the third to fifth day, thus suggesting similarity to the behavior of succulents in continued darkness.

#### EFFECT OF THE LIGHT PERIOD ON ACIDITY RELATIONS IN SHORT-DAY PLANTS

In the study of acidity of the cell sap as affected by the duration of the illumination period four general forms or features of plant development have been included, namely, (1) the sterile vegetative stem; (2) stem

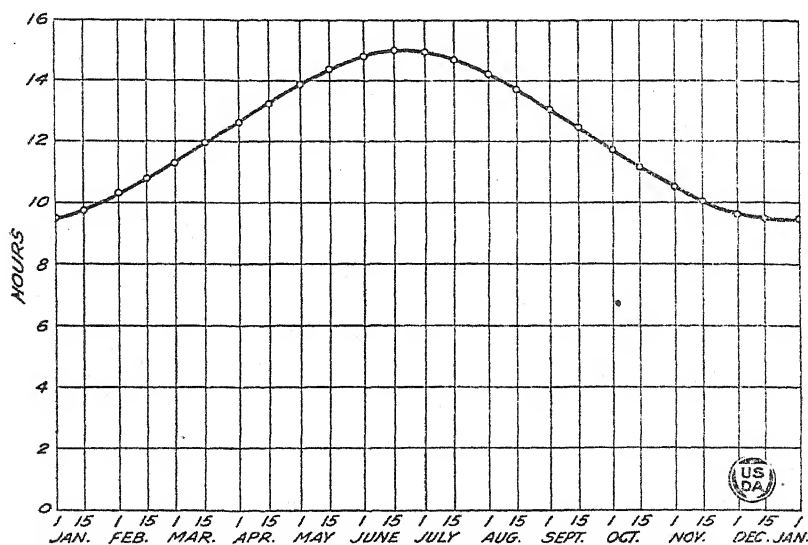


FIG. 1.—Approximate length of day, from sunrise to sunset, for the latitude of Washington, D. C.

elongation accompanied by flowering; (3) the leaf-rosette type of growth, with clearly defined tuberization; (4) the leaf-rosette type of growth, without pronounced tuberization. For comparative purposes the plants may be grown throughout their development under the respective light periods favorable to these different types of activity or at the proper time the plants may be shifted from one light-period to another so as to change the type of development. Both plans have been followed. To facilitate comparison of the artificially regulated daily light periods employed in the experiments with the prevailing natural length of day (sunrise to sunset), the approximate annual range in day length in the latitude of Washington is shown in figure 1.

As already explained, the term "short-day plants" is applied to those species and varieties which tend to produce indeterminate vegetative

stems when exposed to long days but quickly flower when exposed to short days. Late-flowering varieties of *Cosmos bipinnatus* Cav., Biloxi soy beans, the Maryland Mammoth variety of tobacco (*Nicotiana tabacum* L.), and *Tithonia rotundifolia* (Mill) Blake are examples of this group. In this type of plant the height attained is usually proportional to the duration of the daily light period, whether or not flowering occurs. In most cases, however, it is not possible to completely eliminate stem elongation by shortening the light period, so that only the two first-named forms of development need be considered. A rather extreme illustration of these two contrasted forms of development in *Bidens frondosa* L., which behaves in the same manner as *Cosmos bipinnatus*, is shown in plate 1, A.

#### EXPERIMENTS WITH COSMOS BIPINNATUS

Late-flowering white cosmos was planted in boxes on May 2 and had germinated on May 6. One lot of plants received only 10 hours of illumination daily from the beginning of the experiment, a second lot was exposed to the natural length of day up to and including July 11 and thereafter was exposed to 10 hours of illumination daily, while a third lot was exposed to the natural length of day throughout the test. Very small flower buds could be seen on the plants of the first lot by June 1 and on the second lot by July 24, while no flower buds were visible on the third lot of plants till September 19. The hydrogen-ion concentration of the cell sap of the apex and the topmost and basal portions of the stem under the two conditions of illumination is shown in Table I. The material designated as "apex" includes the growing point, the extreme tip of the stem and the attached leaflets. For "top of stem" a section  $1\frac{1}{2}$  to 2 inches long immediately below the apex was used and a section immediately above the ground level of the same length was employed as "base of stem." The data presented in this table include observations at fairly wide intervals for the plants exposed to a 10-hour day from the outset and for the earlier stages of development of the plants exposed to the natural length of day. More frequent observations are given for the plants transferred from the natural length of day to the 10-hour day after considerable growth had already been attained and for later stages of development of the plants under the full length of day of summer. In order to complete the data, a second series of observations was made in such way as to supplement the data of Table I. For this purpose a second planting of the white cosmos was made on June 5 and the seed had germinated on June 8. One series was grown from the beginning under a 10-hour day and a second series was exposed to the full length of day of summer throughout the test. Under the shortened length of day flower buds were found on June 30, but none appeared during the test under the long-day conditions. The observations were made in the same manner as before, and the results are shown in Table II.

TABLE I.—Showing the  $P_H$  value of the cell sap of the apex and upper and lower portions of the stems of late-flowering white cosmos under the natural length of day and under a 10-hour day during the spring and summer months (seed germinated May 6)

Plants exposed to natural length of day.										Plants exposed to a 10-hour day. <sup>a</sup>					
Date of sam- ple.	Hour of sampling.				F <sub>H</sub> value.			Hour of sampling.			F <sub>H</sub> value.				
	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.			
May 19.	11.30 a. m.	12.30 p. m.	12.30 p. m.	6.24	5.83	5.60	12.05 p. m.	1.10 p. m.	1.10 p. m.	6.36	5.70	5.69			
June 2.	12 noon.	12.35 p. m.	1.00 p. m.	6.12	6.28	5.67	11.45 a. m.	12.45 p. m.	1.25 p. m.	6.29	6.17	5.74			
June 9.	12.30 p. m.	1.00 p. m.	1.00 p. m.	5.84	5.80	5.74	11.55 a. m.	1.25 p. m.	1.30 p. m.	b 6.05	c 5.63	5.63			
July 1.	9.35 a. m.	11.15 a. m.	11.15 a. m.	5.33	5.37	5.34	12 noon.	1.55 p. m.	1.55 p. m.	b 5.95	c 5.64	5.71			
July 12.	12.30 p. m.	1.30 p. m.	1.30 p. m.	5.23	5.24	5.40	10.05 a. m.	2.30 p. m.	2.30 p. m.	5.33	5.36	5.41			
July 14.	8.58 a. m.	11.40 a. m.	1.30 p. m.	5.36	5.43	5.33	10.45 a. m.	2.15 p. m.	2.15 p. m.	5.53	5.33	5.32			
July 17.	8.35 a. m.	do.	1.15 p. m.	5.20	5.35	5.16	10.20 a. m.	2.40 p. m.	2.40 p. m.	5.48	5.54	5.36			
July 19.	8.20 a. m.	11.55 a. m.	1.45 p. m.	5.25	5.38	5.36	12.20 p. m.	2.25 p. m.	2.25 p. m.	5.53	5.54	5.39			
July 24.	8.40 a. m.	10.25 a. m.	1.25 p. m.	5.30	5.25	5.35	9.30 a. m.	1.15 p. m.	1.15 p. m.	5.54	5.33	5.39			
Aug. 5.	12.40 p. m.	1.30 p. m.	2.30 p. m.	5.16	5.31	5.32	12.20 p. m.	2.30 p. m.	2.30 p. m.	b 5.99	c 5.68	5.46			
							8.55 a. m.	8.55 a. m.	8.55 a. m.	d 5.27	e 5.43	5.43			

a The data for the period May 20 to June 15, inclusive, relate to plants exposed to the 10-hour day from the beginning of the experiment; the data for the subsequent period relate to plants exposed to the nominal length of day up to and including July 11, and thereafter exposed to the 10-hour day.

*b* Flower buds, only.

c Topmost section of flower bud stem.

**d Newly opened blossoms.**

Topmost section of flowering stem.



TABLE II.—Showing the  $P_H$  value of the cell sap of the apex and the upper and lower portions of the stem of late-flowering white cosmos when grown from germination (June 8) under the natural length of day and under a 10-hour day during the summer months

Date of sam- ple.	Plants exposed to natural length of day.						Date of sam- ple.	Plants exposed to a 10-hour day.					
	Hour of sampling.			P <sub>n</sub> value.				Hour of sampling.			P <sub>n</sub> value.		
	Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.		Apex.	Top of stem.	Base of stem.	Apex.	Top of stem.	Base of stem.
June 19.....	11.45 a. m.	12.35 p. m.	12.35 p. m.	6.46	5.80	5.78	June 20.....	12 noon.....	1 p. m.	1 p. m.	6.26	5.91	5.64
June 22.....	12.30 p. m.	1.35 p. m.	1.35 p. m.	6.52	6.17	5.79	June 23.....	12.50 p. m.	1.50 p. m.	1.50 p. m.	6.46	5.82	5.69
June 26.....	11.45 a. m.	12.40 p. m.	12.40 p. m.	6.46	5.94	5.76	June 27.....	11.45 a. m.	12.45 p. m.	12.45 p. m.	6.24	5.85	5.66
June 29.....	12.35 p. m.	1.40 p. m.	1.40 p. m.	6.36	6.69	5.84	June 30.....	12.05 p. m.	1.10 p. m.	1.10 p. m.	6.22	5.86	5.53
July 6.....	11.40 a. m.	12.35 p. m.	12.35 p. m.	6.18	6.77	5.65	July 3.....	12.35 p. m.	1.25 p. m.	1.25 p. m.	a 6.17	5.76	5.57
July 20.....	12.05 p. m.	12.55 p. m.	1.55 p. m.	5.59	5.69	5.65	July 12.....	12.15 p. m.	1.35 p. m.	2.40 p. m.	b 6.06	5.67	5.59
July 28.....	8.40 a. m.	9.30 a. m.	11.45 a. m.	5.27	5.42	5.49	July 13.....	12.40 p. m.	1.30 p. m.	1.50 p. m.	b 5.82	5.62	5.57
							July 27.....	12.15 p. m.	12.15 p. m.	1.50 p. m.	c 5.26	d 5.48	5.57
							July 28.....	9.30 a. m.			e 5.58		

a Small flower buds rejected in sampling.

b Flower buds only.

c Fully opened blossoms.

d Topmost section of flower stems.

e Seed heads, in green stage.

The results recorded in Tables I and II, taken as a whole, are quite consistent and leave no doubt as to the conclusions to be drawn. There is a striking difference in the course of the acidity relations during the progress of development of the plant under the long-day and short-day conditions and these differences are clearly associated with differences in form of expression caused by the two conditions of illumination. The seat of maximum change in acidity is found in the region of the growing point. Under the longer daily illumination period the plants at the age of 2 to 3 weeks show a relatively low acidity, particularly in the apical structures, but with continued elongation of the stem there is a marked, progressive increase in acidity, the  $P_H$  number for the apex declining from a value well above 6 to approximately 5.2, at which point it remains comparatively stationary (fig. 2). The advanced stage of vegetative development of the stem, therefore, is characterized by relatively high acidity of the apex as compared with early stages of development. The

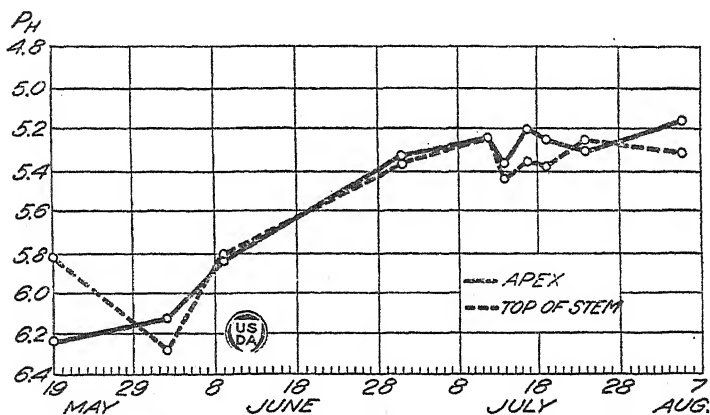


FIG. 2.—Showing the characteristic progressive increase in active acidity in the sap of the apical tissues of late-flowering cosmos when exposed to the long days of summer (Table I). Cosmos is a typical short-day plant and in this group indeterminate elongation of the vegetative stem under a long-day exposure is associated with a progressive increase in active acidity in the region of the apex. The relatively high active acidity of the advanced vegetative stage is maintained during the period of active vegetative growth.

portion of the stem immediately below the apex shows greater acidity than the apex in earlier stages of development, but as growth proceeds this relationship is reversed. The base of the stem is initially the most acid portion of the plant, but shows only moderate subsequent change, and hence in later stages of growth tends to become the least acid part of the plant (fig. 3).

When the plant is exposed to a 10-hour day from date of germination, which has the effect of promptly initiating flowering, there is an appreciable but transitory decrease in hydrogen-ion concentration in the tissue fluids of the apex, which takes place about 2 weeks after germination. The probable significance of this phenomenon is later referred to more fully (p. 148.) This temporary decrease is followed by increase in acidity till the  $P_H$  number of approximately 6 is reached, a level of acidity which is characteristic of the flowering condition and represents the acidity of the young flower buds themselves. The flower buds and adjacent tissues increase in acidity till the unfolding of the blossom is completed. This latter stage marks the culmination of the increase in acidity of the repro-

ductive structure, and it is interesting to note that the  $P_H$  value is the same as that finally attained in the apex in advanced vegetative development (fig. 4). There is but little change in acidity in upper and lower portions of the stem except that after flowering has been initiated the former increases somewhat in acidity. As compared with the action of long days, the effect of the 10-hour day on the plant during the first 3 weeks of growth following germination is to increase the acidity of the cell sap, except for the above-mentioned temporary decrease in acidity under the short-day exposure.

After the acidity relations characteristic of the advanced stage of vegetative development have become established abrupt change from long-day to short-day conditions results in decided decrease in acidity of the apex by the fifth day, but this change is not apparent by the second day after the transfer had been made (fig. 5). Comparing acidity relations under the short-day exposure with those under the long-day exposure, it is evident that at the time the flowering condition is attained under the

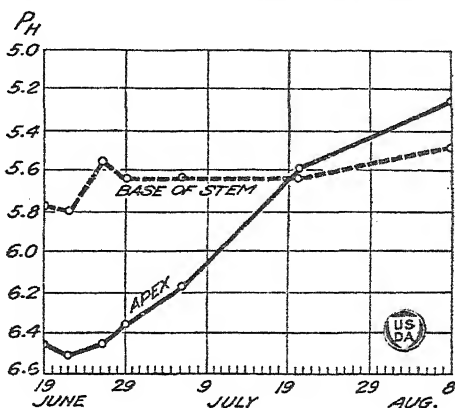


FIG. 3.—Showing the active-acidity relations in the apex and base of the stem in late-flowering cosmos when exposed to the long days of summer (Table II). In contrast with the sharp progressive increase in active acidity of the apex in this group of plants when exposed to long days there is but little change in the active acidity of the base of the stem. As a result, advance in vegetative development leads to a reversal in relative acidity of the apex and basal portion of the stem.

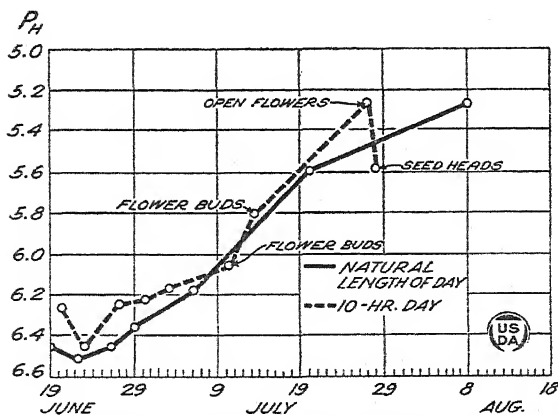


FIG. 4.—Showing the course in active acidity of the apex in late-flowering cosmos when exposed to the relatively long days of summer and to a 10-hour day, respectively, from date of germination (Table II). In short-day plants of this type exposure to a 10-hour day promptly initiates flowering. Under these conditions there is during the early stage of development a well-defined temporary decrease in active acidity (compare fig. 10), probably indicative of initiation of flowering. There is then a progressive increase in acidity, culminating in the freshly open blooms and finally a decrease in the developing seed head or fruit.

moment the unfolding of the blossom is completed the acidity relations are quite similar to those seen in the advanced stage of vegetative development.

former the acidity gradient of the axis is the reverse of that found in the advanced stage of vegetative development under the latter exposure, but is similar to the gradient found in the earlier stages of growth under the long-day exposure. As a matter of fact, the plants exposed to the longer daily illumination period show at a certain stage acidity relations quite similar to those characteristic of the flowering condition, as shown in the data of July 6 in Table II. On the

other hand, at the mo-

TABLE III.—Showing the  $P_H$  value of the cell sap of the different organs in late-flowering cosmos when exposed to the natural length of day and to a 10-hour day during the summer months (seed germinated May 24).

Date of observation and part of plant used.	Plants exposed to natural length of day from date of germination.		Plants exposed to natural length of day from date of germination to June 28 and thereafter exposed to a 10-hour day.	
	Hour of sampling.	$P_H$ value.	Hour of sampling.	$P_H$ value.
July 12:				
Apex.....	9.15 a. m....	5.23	9.15 a. m....	<sup>a</sup> 5.56
Top leaves.....	1.35 p. m....	5.22	11.30 a. m....	5.48
Lower leaves.....	...do.....	5.59	...do.....	5.81
July 15 (and 17): <sup>b</sup>				
Apex.....	9.05 a. m....	5.18	9.15 a. m....	<sup>a</sup> 5.84
Top of stem.....	...do.....	5.30	...do.....	5.47
Middle of stem.....	11.15 a. m....	5.43	11.25 a. m....	5.47
Base of stem.....	...do.....	5.46	...do.....	5.43
Roots (fibrous).....	1.50 p. m....	5.46	2 p. m.....	5.46
July 20:				
Top leaves.....	2 p. m.....	4.98	11.30 a. m....	5.15
Lower leaves.....	...do.....	5.65	...do.....	5.79
July 21:				
Apex.....	11.20 a. m....	4.98	9.15 a. m....	<sup>a</sup> 5.63
Top of stem.....	...do.....	5.14	...do.....	5.49

<sup>a</sup> Flower buds only.

<sup>b</sup> Observations of plants under the 10-hour day conditions were made on July 17.

To obtain further information on the relative acidity of the different organs of the plant as affected by the length of day, observations were made on plantings of cosmos which germinated May 24. One series was

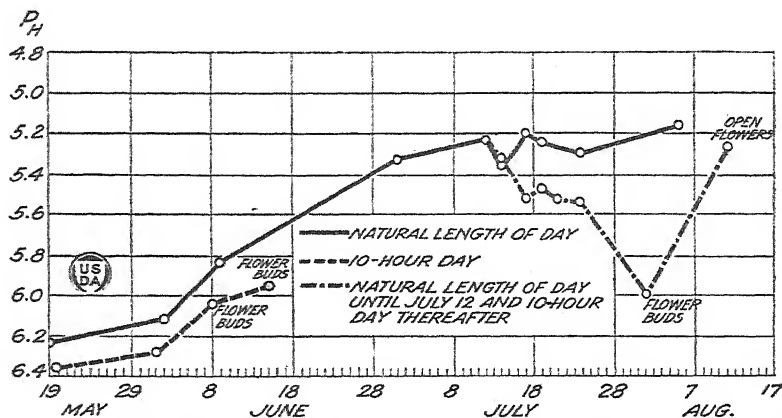


FIG. 5.—Showing the effect of abrupt change in light exposure from the long days of summer to a 10-hour day, on the active acidity of the sap in the apex of late-flowering cosmos (Table I). There is a prompt drop in active acidity to a level which is far below that characteristic of the advanced vegetative stage and the same as that exhibited in plants exposed to a 10-hour day from the outset. Here, again (compare Fig. 4), the active acidity of the open blossom is practically identical with that of the apical bud in the advanced vegetative stage which results from a long-day exposure.

exposed to the natural length of day throughout the test and a second series was transferred from the full day length to a 10-hour day on June 28. Flower buds were found on the latter series on July 8. The data obtained are given in Table III. For the sample of top leaves the three

uppermost were used; for lower leaves the oldest available were taken, some of which had begun to yellow. The upper and lower leaves show much the same acidity relations as the corresponding portions of the axis and in all cases the acidity is lowest under the 10-hour day. The acidity of the fibrous roots is about the same as that of the base of the stem.

During the short days of winter electric light of intensity as low as 5 foot-candles used to prolong the day length is effective in preventing flowering in cosmos, and the observations recorded in Table IV (A) indicate that the acidity relations characteristic of the more advanced vegetative condition are thereby maintained. After flowering has been induced in cosmos by exposure to short-day conditions a return to the vegetative condition, involving phenomena of rejuvenescence, is eventually effected by exposure of the plant to long-day conditions. Cosmos which was planted in the greenhouse March 11 formed flower buds in response to the short seasonal length of day. The first blossom opened May 8. By May 21 it was evident that as a result of the increasing length of day the plants were passing definitely into the vegetative condition. Observations on these plants are recorded in Table IV (B). Seedlings of cosmos which germinated May 15 under a 10-hour day were showing flower buds by June 3. On June 13 these plants were placed permanently out of doors. By June 28 evidence could be seen of the change to the vegetative condition, though flowering had not entirely ceased. Transition stages in this type of rejuvenescence are shown in Plate 1, B. Observations on these plants are given in Table IV (B). It is obvious that change from short-day to long-day conditions after flowering has been initiated results in establishing the relatively high acidity of the apical structures which is characteristic of the advanced vegetative condition.

#### EXPERIMENTS WITH BILOXI SOY BEANS

The type of growth in soy beans differs markedly from that in cosmos in that stem elongation is decidedly reduced, there is much branching, and development of foliage in proportion to stem is greater. Late maturing varieties of soy beans, however, show much the same responses to length of day as does late cosmos. For study of acidity relations the young, topmost leaves averaging 1 to 1½ inches in length were commonly used. As the flowering condition is approached the formation of new leaves is checked, so that in such cases, while the leaves taken for study were of the usual size, they were probably somewhat older than those gathered during the more active vegetative period. In a series of observations on plants growing in the field during the months of June and July, which will not be reported here in detail, it was found that the acidity of the sap from the young, topmost leaves remained close to the  $P_H$  value 6.50 from the earliest stages of growth up to the approach of the flowering condition, while thereafter the acidity increased up to a maximum of  $P_H$  5.97 after seed pods had appeared. Larger, more mature leaves showed a somewhat higher acidity. The acidity gradient of the stem and root during the vegetative period was found to be quite similar to that in cosmos. Typical  $P_H$  values found for top and base of stem and fibrous roots are 5.97, 6.17, and 6.46, respectively. As flowering buds came into evidence there was a temporary rise in  $P_H$  value in the top of stem to about 6.25, followed by a return toward previous values as the flowers unfolded.

TABLE IV.—Observations on late-flowering cosmos, showing: (A) the effectiveness of low-intensity electric light as a supplement to daylight in establishing the high hydrogen-ion concentration of the cell sap which is associated with the advanced vegetative condition, and (B) the rise in hydrogen-ion concentration of the upper parts of the plant in the return from the flowering to the vegetative condition (rejuvenescence) which results from exposure to increased length of day

A				B			
Planted Nov. 1 (and Jan. 5) in the greenhouse and exposed to natural daylight supplemented with electric light to furnish 18 hours of illumination daily.				Planted Mar. 11 in greenhouse and exposed to natural increase in length of day.			
Date and hour of sampling.	Part of plant used.	P <sub>H</sub> value.		Date and hour of sampling.	Part of plant used.	P <sub>H</sub> value.	
Apr. 15, 9:30 a. m.	Topmost leaves.	5.17		June 10, 12:30 p. m.	Flower buds.	6.10	
Apr. 20, 11:40 a. m.	do.	5.06		June 21, 12:30 p. m.	do.	5.93	
May 6, 1:45 p. m.	do.	5.21		June 28, 12 noon.	Open blossoms.	5.25	
May 7, 12:35 p. m.	Apex.	5.30		Aug. 11, 10:45 a. m.	Apex (vegetative).	5.24	
May 22, 1:05 p. m.	Topmost leaves.	5.17					

<sup>a</sup> Planted Jan. 5.

To study further the effect of the light period on acidity, plantings were made in six metal buckets of 12-quart capacity on June 30, and the seedlings were above ground on July 3. One series of three buckets remained out of doors throughout the test, while a second series of three buckets was permanently transferred from the full length of day to a 10-hour day on August 5. The latter series began flowering August 29. The former series began flowering September 15, although flower buds could be seen a week earlier. The results of the observations are shown in Table V and figure 10. These studies were only begun shortly before the plants passed into the flowering condition as a result of the natural decrease in length of day, for it is known that a minimum period of at least 3 weeks is required for the unfolding of the blossom after the plant comes under the influence of a favorable day length. It will be seen that under the natural length of day there is a decided decrease in acidity for about one week ending August 19, immediately followed by a sharp increase which continues progressively till after flowering has begun. Abrupt shortening of the daily light period to 10 hours had somewhat reduced the hydrogen-ion concentration at the end of 48 hours, but the full effect of the change did not come about till the end of 5 or 6 days. Thereafter there was a sharp progressive increase in acidity just as under the natural decrease in length of day. The effect of the 10-hour day, however, was to accentuate and to hasten these changes in acidity. Biloxi soy beans thus closely resemble cosmos in acidity relations except that the relatively reduced stem elongation characteristic of the soy beans in the vegetative condition is associated with a decidedly lower maximum acidity of the apical structures than is attained in cosmos, in which marked elongation of the axis takes place.

TABLE V.—Showing the  $P_H$  value of the cell sap of the young, topmost leaves of Biloxi soy beans grown in buckets when exposed to the natural change in length of day in late summer and fall and when abruptly transferred from these conditions of illumination to a 10-hour day.

Time at which sample was taken.		Plants exposed to natural length of day throughout the test.	Plants permanently transferred from the natural length of day to a 10-hour day on Aug. 5.
Date.	Hour.	$P_H$ value.	$P_H$ value.
Aug. 7.....	9.30 a. m.....	6.28	6.44
Aug. 9.....	9.20 a. m.....	6.31	6.52
Aug. 11.....	9.30 a. m.....	6.41	6.95
Aug. 13.....	9.10 a. m.....	6.56	7.00
Aug. 16.....	9.20 a. m.....	6.36	6.60
Aug. 19.....	9.40 a. m.....	6.70	6.51
Aug. 24.....	11.15 a. m.....	6.33	6.36
Aug. 31.....	10.05 a. m.....	6.32	.....
Sept. 1.....	12.15 p. m.....	.....	6.21
Sept. 7.....	11.25 a. m.....	6.29	6.27
Sept. 16.....	9.45 a. m.....	6.27	6.07
Sept. 21.....	11 a. m.....	6.08	.....

The preceding experiment was repeated with two rows of Biloxi soy beans planted in the field which had germinated May 19 and 21, respectively. Beginning July 24, a portion of the plants were allowed to receive only 10 hours of light daily, this being accomplished by placing over the plants a ventilated, light-proof box which could be opened and closed as desired. In this case the exposure to the shortened day length was not begun until just as the plants were passing over into the flowering condition as a result of the natural decrease in length of day. Flower buds could first be seen on August 10 under the 10-hour day and on August 22 under the natural length of day. The transition from the vegetative to the reproductive stage occurred considerably earlier than in the preceding experiment because the plants had been seeded much earlier. The results of the observations, presented in Table VI, are even more sharply defined than those of the preceding test with respect to the initial decrease in acidity, and bring out clearly the same general relationships as to the course of changes in acidity. This test emphasizes the fact that under the conditions important changes in acidity as a result of abrupt shortening of the day length do not occur till after the lapse of 2 or 3 days.

TABLE VI.—Showing the  $P_H$  value of the cell sap of the young, topmost leaves of Biloxi soy beans growing in the field when exposed to the natural change in length of day in summer, and when the daily light period was abruptly changed from the natural daylight period of summer to a 10-hour period of illumination

Date of sample.	Plants exposed to natural change in length of day throughout the test.		Plants exposed to natural change in length of day till July 24 and thereafter exposed to a 10-hour day.	
	Hour of sampling.	$P_H$ value.	Hour of sampling.	$P_H$ value.
	a. m.		a. m.	
July 22.....	9. 50	6. 37		
July 25.....	9. 00	6. 38	10. 20	6. 37
July 27.....	9. 15	6. 80	10. 25	6. 57
July 29.....	8. 50	6. 27	10. 30	6. 39
Aug. 1.....	9. 15	6. 11	10. 30	6. 28
Aug. 4.....	10. 35	6. 20	9. 05	6. 20
Aug. 7.....	9. 25	6. 07	10. 45	6. 01
Aug. 10.....	10. 30	6. 20	9. 10	6. 03
Aug. 14.....	9. 35	6. 18	11. 00	6. 16
Aug. 17.....	10. 15	6. 04	9. 00	6. 08
Aug. 22.....	10. 00	6. 24		
Aug. 25.....	9. 45	6. 07		
Aug. 30.....	9. 50	6. 10		
Sept. 8.....	9. 45	6. 06		

Studies were made on acidity relations in the developing seed pods of Peking soy beans, which had germinated June 27, as representing the final features of reproductive activity. In the earlier stages of development the seed pods in their entirety were necessarily taken, but as soon as the seeds had attained sufficient size these were separated from the hulls. The samples were collected at 1.30 to 2.30 p. m. For analysis the seeds in the more mature stages were ground with distilled water. The results are shown in Table VII. The hulls, constituting the vegetative portion of the fruit, increase in acidity as they develop, while the



seeds show a much lower and decreasing acidity till about the time maximum size is attained. During the later stages of ripening the seeds appear to increase somewhat in acidity.

TABLE VII.—Showing the  $P_H$  value of cell sap of seed pods and seeds in the Peking variety of soy beans at different stages of development

Date of sample.	Material.	$P_H$ value.
Aug. 31.....	Entire seed pods, less than $\frac{1}{2}$ inch long.....	6.29
Sept. 1.....	Entire seed pods, about 1 inch long.....	6.09
Do.....	Entire seed pods, about $1\frac{1}{2}$ inches long.....	6.10
Sept. 9.....	Entire seed pods, about $1\frac{3}{4}$ inches long.....	6.24
Sept. 19.....	Hulls only, $1\frac{1}{2}$ to $1\frac{3}{4}$ inches long.....	5.94
	Seeds only.....	6.63
Sept. 28.....	Hulls only, $1\frac{1}{2}$ to $1\frac{3}{4}$ inches long.....	5.91
	Seeds only, just beginning to turn red.....	6.83
Oct. 5.....	Seeds only, full size, seed coats red in color.....	6.63
Oct. 7.....	Seeds only, black, nearly dry.....	6.33
Oct. 28.....	Seeds only, dry and mature.....	6.34

#### EXPERIMENTS WITH MARYLAND MAMMOTH TOBACCO

This variety of tobacco behaves like the late varieties of cosmos and soy beans in response to length of day, and observations made on acidity relations gave results similar to those already presented for cosmos and soy beans. Typical  $P_H$  values obtained for the terminal bud and the topmost and basal portions of the stem, respectively, are 5.25, 5.53, and 5.30 during the vegetative stage of the plant, and 5.46, 5.51, and 5.29 just after the flower bud became visible. The acidity gradients of the stem under the two conditions are similar to those obtained for cosmos, though not quite so pronounced.

#### EXPERIMENTS WITH TITHONIA ROTUNDIFOLIA (MILL.) BLAKE

This tropical weed attains giant proportions without flowering when grown during the summer months in the latitude of Washington, but flowering is readily induced by shortening the length of day. Studies were made on the acidity of the cell sap as affected by the daily light period. Seed were planted May 16 and the seedlings transferred to boxes on June 20. One series was exposed to the natural length of day throughout the test, a second series was permanently transferred from the natural length of day to a 10-hour day on July 12, and a third series was similarly transferred to a 10-hour day on August 17. Determinations of hydrogen-ion concentration in the topmost and basal portions of the stem were made, using for the purpose sections  $1\frac{1}{2}$  to 2 inches in length. Under the natural length of day no flower buds could be seen as late as October 9, while they were first visible July 26 in the plants transferred to the 10-hour day on July 12 and were first seen on September 6 in the plants similarly transferred on August 17. The results of the acidity measurements are summarized in Table VIII. Under the long days of summer the acidity of the stem increased steadily up to August 22 but thereafter the decreasing length of day caused progressive decrease in acidity (fig. 6). The first transfer of plants to the short day was made before the acidity had increased to that associated with the advanced vegetative condition, and

under these circumstances a slight increase in acidity as compared with the plants remaining under the full length of day was found on the sixth day after the transfer. A week later, however, the plants exposed to the longer daylight period had become the more acid. Transfer of the plants to the 10-hour day after the advanced vegetative stage had been reached had the effect of bringing about a transitory decrease in acidity about the fifth day after the transfer had been made. This was soon followed by a moderate increase in acidity prior to the appearance of the flower bud.

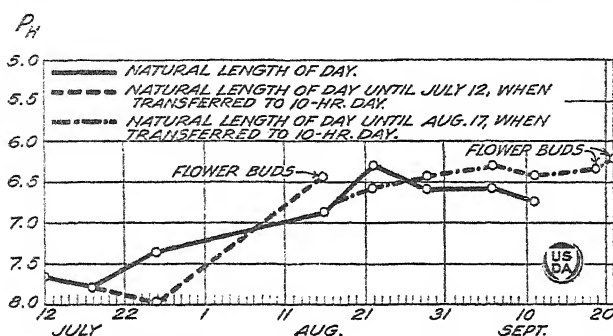


FIG. 6.—Showing the active-acidity relations in the uppermost portion of the stem in *Tithonia rotundifolia* when exposed to the long days of summer and to a 10-hour day, respectively (Table VIII). These relationships are very similar to those found in cosmos. Abrupt decrease in the light period causes a decrease in active acidity, followed by rise during the development of the flower bud.

TABLE VIII.—Showing the  $P_H$  value of cell sap in topmost and basal portions of the stem in *Tithonia rotundifolia* as affected by the duration of the daily illumination period

Date of sample.	Plants exposed to natural length of day.				Plants transferred from the natural length of day to a 10-hour day. <sup>a</sup>			
	Hour of sampling.		$P_H$ value.		Hour of sampling.		$P_H$ value.	
	Top of stem.	Base of stem.	Top of stem.	Base of stem.	Top of stem.	Base of stem.	Top of stem.	Base of stem.
July 12.....	8.45 a. m....	10.20 a. m....	7.61	6.46	.....	.....	.....	.....
July 18.....	11.55 a. m....	2.25 p. m....	7.78	6.78	1.10 p. m....	3.25 p. m....	7.79	6.26
July 26.....	9.05 a. m....	12.20 p. m....	7.34	5.96	10.50 a. m....	1.35 p. m....	7.96	6.04
Aug. 16.....	9.10 a. m....	1.35 p. m....	6.87	5.63	10.25 a. m....	3 p. m....	<sup>b</sup> 6.44	5.74
Do.....	.....	.....	.....	.....	12.20 p. m....	.....	<sup>c</sup> 6.34	.....
Aug. 22.....	12.10 p. m....	.....	6.30	.....	1.25 p. m....	.....	6.57	.....
Aug. 29.....	1.20 p. m....	.....	6.60	.....	12.20 p. m....	.....	6.46	.....
Sept. 6.....	12.25 p. m....	.....	6.59	.....	1.30 p. m....	.....	6.32	.....
Sept. 11.....	2.25 p. m....	.....	6.76	.....	1.20 p. m....	.....	6.43	.....
Sept. 19.....	.....	.....	.....	.....	3 p. m....	.....	<sup>b</sup> 6.36	.....
Sept. 21.....	.....	.....	.....	.....	1 p. m....	.....	<sup>b</sup> 6.23	.....

<sup>a</sup> The data for the period July 18 to Aug. 16, inclusive, relate to plants permanently transferred from the natural length of day to a 10-hour day on July 12; subsequent data relate to plants similarly transferred on Aug. 17.

<sup>b</sup> Flower buds about one-half inch in diameter.

<sup>c</sup> Upper portion of flower bud stem.

#### EFFECT OF THE LIGHT PERIOD ON ACIDITY RELATIONS IN LONG-DAY PLANTS

In plants of this group exposure to relatively long days results in stem elongation, which is invariably coupled with flowering; that is, the condition of indeterminate elongation of the sterile vegetative stem is wanting. Exposure to relatively short days tends to inhibit stem

elongation and flowering, thus resulting in the leaf-rosette type of development, either with or without tuberization. Radish (*Raphanus sativus* L.) may be taken as an example of this type in which a short daily illumination period results in marked tuberization without stem elongation, while *Rudbeckia bicolor* L. may be taken to represent the type in which tuberization does not result under these conditions.

#### EXPERIMENTS WITH SUMMER RADISH

Radish of the horticultural variety Scarlet Globe was planted in wooden boxes and galvanized-iron cans in the greenhouse on September 29 and had germinated on October 3. One lot of plants was exposed to the natural length of day while the other lot received, in addition, illumination from 50-watt electric bulbs without reflectors, placed about 18 inches above the plants. The electric lights were turned on at sunset and turned off at midnight, the plants thus receiving about 18 hours of illumination daily. By December 6 development of the primary axis had begun in 46 individuals, or 82 per cent, of the plants exposed to the 18-hour day, and under the natural length of day 9 plants, or 17 per cent, of the total were developing a stem. Of the two lots the plants under the longer illumination period were much the taller. While the natural length of day of winter at Washington is not short enough to permanently inhibit stem elongation in this radish, the process is much delayed and restricted.

Results of observations on the hydrogen-ion concentration of the sap of the plants under the two conditions are shown in Table IX. For the leaf material the entire midribs of the larger leaves were used after removal of the lamina. In sampling the tuber or thickened root approximately the upper third was taken for the first sample and for the second sample a central horizontal section constituting approximately a third of the total was used, the lower third with tap root being rejected. In sampling the primary stem, in the case of the plants exposed to the longer illumination period, the topmost section of 1 inch in length and a section one-half inch in length immediately above the thickened root were used. For the samples taken on December 15 plants were used which had developed stems 9 inches high, while the plants used four days later had stems only 6 inches high. The plants used January 4 had developed stems 32 inches high which were showing flower buds. It may be added at this point that unopened flower buds collected January 5 from somewhat further advanced plants showing open blossoms gave a  $P_H$  reading of 5.51. In the case of plants exposed to the natural length of day only those were used which showed no evidence of developing flowering stems. In this instance the acidity values corresponding to those of the top of the stem in the plants under the lengthened light exposure relate to the tissues in the region of the growing point immediately above the thickened root after removal of the leaves of the rosette. The data in Table IX show that under the relatively short daily illumination period, which is unfavorable for stem elongation, the acidity of the plant as a whole is relatively low and is lower in the region of the growing point than elsewhere. Under the longer light period the acidity of the plant is higher, especially in the upper part of the stem, which increases in acidity as growth proceeds. It may be added here that while the average weight of tops of the plants under the longer daily illumination was much greater, the average weight of roots was decidedly less than the corresponding

TABLE IX.—Showing the  $P_n$  value of the cell sap of summer radish when exposed to the natural length of day of winter and to the natural daylight period supplemented with electric light to give 18 hours total illumination daily

Part of plant used.	Plants exposed to natural length of day of winter.						Plants exposed to natural daylight supplemented with electric light so as to furnish 18 hours of illumination daily.					
	Dec. 14.		Dec. 17.		Jan. 6.		Dec. 15.		Dec. 19.		Jan. 4.	
	Hour of sampling.	$P_n$ value.	Hour of sampling.	$P_n$ value.	Hour of sampling.	$P_n$ value.	Hour of sampling.	$P_n$ value.	Hour of sampling.	$P_n$ value.	Hour of sampling.	$P_n$ value.
Top of primary stem <sup>a</sup> .....	11:20 a. m. ....	6.38	11:15 a. m. ....	6.55	10:45 a. m. ....	6.49	11:20 a. m. ....	5.94	11 a. m. ....	6.25	11:20 a. m. ....	5.27
Base of primary stem.....	9:55 a. m. ....	6.68	10:05 a. m. ....	6.66	9:45 a. m. ....	5.96	10 a. m. ....	6.14	.....do.....	6.38	1:10 p. m. ....	6.33
Stem of leaf.....	1:15 p. m. ....	6.44	1 p. m. ....	6.40	12:40 p. m. ....	6.49	2:05 p. m. ....	5.92	9:40 a. m. ....	5.87	10:10 a. m. ....	5.80
Upper portion of tuber.....	.....do.....	6.61	.....do.....	5.85	.....do.....	5.72	.....do.....	6.10	1:45 p. m. ....	6.29	2 p. m. ....	6.06
Central section of tuber.....								5.97	.....do.....	5.96	.....do.....	6.03

<sup>a</sup> In case of plants exposed to natural length of day samples consisted of the tissues in the region of the growing point situated immediately above the thickened root, after removal of the leaves, there having been no considerable elongation of the axis.

parts of the plants exposed to the shorter illumination period. The above results of the studies on acidity relations were confirmed by observations on plants exposed to the full length of day of late spring and summer in comparison with plants exposed to a 10-hour day, but it seems unnecessary to present the data in detail. In these latter experiments the plants germinated April 13. As late as June 7 the very young leaves of the rosettes in the plants exposed to a 10-hour day showed a  $P_H$  reading of 6.06 while the tops of the stems of the plants exposed to the natural length of day (nearly 15 hours), which were showing young flower buds, gave a reading of 5.57. By June 26 stem elongation was beginning in the plants under the 10-hour day and the tops of the stems showed a  $P_H$  reading of 5.67.

#### EXPERIMENTS WITH *RUDBECKIA BICOLOR*.

Seeds of this garden ornamental were planted in the greenhouse December 19 and on January 27 the seedlings were transplanted in flats. On February 6 one lot of the small seedlings was transferred from the natural length of day to a 7-hour day. A second lot was exposed to an 18-hour daily illumination, a 100-watt electric bulb being placed 2 feet above the plants and the current turned on at sunset and turned off at midnight each day. On March 22 a portion of the plants which had been exposed to the 7-hour day were permanently transferred to the 18-hour day. Under the 7-hour day stem elongation was indefinitely inhibited, but under the 18-hour day development of the axis had begun within 2 weeks. Growth characteristics under the two light conditions are shown in plate 2, A. Of the plants under the 7-hour day, only the leaves, of course, were available for acidity studies. In sampling, all sound leaves excepting the smallest individual of the rosette were used in both the long-day and the short-day series of plants. The leaves of the plants exposed to the 18-hour day were decidedly longer than those of the plants under the 7-hour day. In sampling the stems of the plants under the 18-hour light period the entire stem was used when the length was less than 4 inches. In all other cases 2-inch sections were used. In sampling the flower bud the involucre was rejected. The results of the observations are summarized in part in Table X. Additional observations which need not be reported in detail have confirmed the data here given. It may be added that samples of open blossoms and upper and lower portions of stems collected May 10 from plants which had been exposed to the 18-hour light period from February 5 gave  $P_H$  readings of 5.55, 5.41, and 5.68, respectively.

The acidity relations of the leaf in *Rudbeckia* differ somewhat from those in radish under the two conditions of illumination. In radish the higher acidity occurred under the longer light period, while the reverse was true in *Rudbeckia*. Again, under the influence of the longer light period maximum acidity of the stem in radish is found in the upper portion even before the appearance of the flower buds. In *Rudbeckia* the topmost portion of the stem is consistently less acid than the basal portion up to and including the period of flower formation (fig. 7). This relationship is not reversed till the blossom has unfolded. In both radish and *Rudbeckia*, however, the acidity of the upper portion of the stem increases progressively prior to the appearance of flower buds, although in both the increase is more pronounced in subsequent development.

TABLE X.—Showing the  $P_H$  value of the cell sap of *Rudbeckia bicolor* exposed to a 7-hour day and an 18-hour day

Date of sample.	Plants exposed to a 7-hour day.		Plants exposed to an 18-hour day. <sup>a</sup>						
	Hour of sampling leaf.	P <sub>H</sub> value of leaf.	Height of stem.	Hour of sampling.			P <sub>H</sub> values.		
				Leaf.	Top of stem.	Base of stem.	Leaf.	Top of stem.	Base of stem.
Feb. 24.....	12.40 p. m.	6.62	<i>Inches.</i> 1½	2 p. m.	3 p. m.		6.93	<i>b</i> 7.69	
Do.....			2½		3.40 p. m.			<i>b</i> 7.32	
Feb. 26.....	10.05 a. m.	6.61	1½	11.10 a. m.	2.05 p. m.		6.94	<i>b</i> 7.43	7.30
Do.....			4		2 p. m.	3.10 p. m.		7.89	
Mar. 3.....			6		10.30 a. m.	11.50 a. m.		7.72	7.03
Mar. 10.....			10		11.40.	12.55 p. m.		7.66	7.04
Mar. 19.....			17	12.40 p. m.	1.40 p. m.	3.40 p. m.	<i>c</i> 6.48	7.24	6.55
Mar. 21.....	10.10 a. m.	6.58							
Apr. 12.....			7		9.55 a. m.	11.10 a. m.		7.17	5.91
Apr. 23.....			18½	11.25 a. m.	12.55 p. m.	1.55 p. m.	<i>c</i> 6.37	6.63	5.82
Apr. 27.....			20	9.50 a. m.	10.55 a. m.	12.45 p. m.	<i>c</i> 6.01	6.27	5.79

<sup>a</sup> The data for the period Feb. 24 to Mar. 19, inclusive, relate to plants exposed to 18 hours of light daily from the beginning of the experiment; the data for the period Apr. 12 to 27, inclusive, relate to plants exposed to the 7-hour day till Mar. 22 and thereafter exposed to the 18-hour day.

<sup>b</sup> Entire stem.

<sup>c</sup> Flower buds.

It is of considerable interest to compare the acidity relations in *Rudbeckia*, as representative of the group of long-day plants which do not ordinarily become tuberized, with the acidity of the short-day plants.

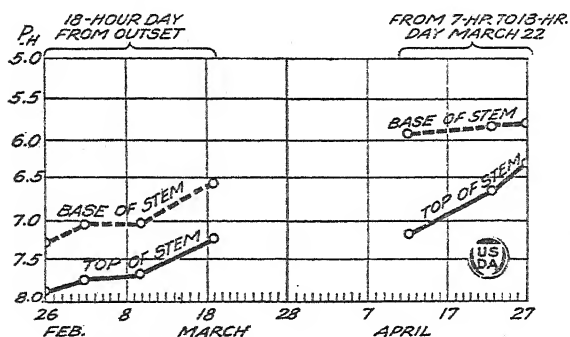


FIG. 7.—Showing the effect of abrupt increase in the daily light period on the active acidity of the cell sap in the stem of *Rudbeckia bicolor*, which is a typical long-day plant (Table X). Increase in the light period from 7 hours to 18 hours initiates elongation of the axis which is followed by flowering. In this group of plants the upper portion of the stem is lower in active acidity than is the base, a condition which is found in short-day plants only during the early stages of development and in the initial stages of reproductive activity. Thus, in the typical long-day plants the level of active acidity characteristic of the flowering stage is attained by an increase rather than a decrease in acidity of the stem apex.

lar. There is in all cases an increase in acidity of the stem till the flower bud appears. The flower buds themselves increase in acidity as growth proceeds, maximum acidity being found in the unfolded blossom. In distribution of acidity the upper portion of the stem is in all cases less acid than the lower portion, in contrast with conditions in the short-day plants when exposed to long days. Radish, as representative of the group of long-day plants which commonly become tuberized under short days, seems to differ somewhat from short-day plants (when exposed to short days) and the *Rudbeckia* group of long-day plants in distribution of acidity in the stem, particularly in the earlier stages of stem development.

The course of development of long-day plants when exposed to relatively long days is more or less similar to that of short-day plants when exposed to relatively short days. By comparing the acidity in the stem of *Rudbeckia* under the 18-hour day (Table X) with that of *cosmos* (Table II) and *Tithonia* (Table VIII) under a 10-hour day it will be apparent that in general the acidity relations are very similar.

## ACIDITY RELATIONS IN PLANTS OCCUPYING AN INTERMEDIATE POSITION BETWEEN THE MORE TYPICAL SHORT-DAY AND LONG-DAY PLANTS

There is a group of plants which may be regarded as standing between typical short-day and long-day plants in their response to differences in length of day. In these plants the height attained increases with increase in the daily illumination period, but the condition of indeterminate elongation of the vegetative stem is not readily attained under relatively long days, nor is stem elongation readily and completely inhibited by exposure to short days. *Helianthus annuus* L. is a representative of this group. The range in length of day during the open growing season at Washington greatly affects the height attained by this sunflower but does not materially influence the time required for flowering. On the other hand, a more extreme change in the light period causes appreciable change in time of flowering and, of course, further accentuates differences in height of the stem. The effect of the light period on the height of the plant is shown in plate 2, B.

A series of plantings of sunflower were made at intervals of two weeks through the summer months. Observations were made on heights attained by the plants, the dates of flowering and the hydrogen-ion concentration of the cell sap at different stages of development. The data on height attained and time of flowering are shown in Table XI. The maximum height was reached by the first two plantings, but unfortunately exact data were not secured on these plantings. The plantings made after August 25 failed to flower because of onset of cold weather.

TABLE XI.—Number of days from germination to flowering and final height attained by *Helianthus annuus* when planted at two-week intervals during the summer months

Date planted.	Date of germination.	Date of first open blossom.	Number of days from germination to flowering.	Average height of plants at time of flowering.
				Inches.
June 15.....	June 29.....	Aug. 19.....	51	.....
June 29.....	July 5.....	do.....	45	.....
July 13.....	July 19.....	Sept. 6.....	49	66
July 27.....	Aug. 2.....	Sept. 19.....	48	60
Aug. 11.....	Aug. 18.....	Oct. 2.....	45	50
Aug. 25.....	Sept. 2.....	Oct. 24.....	52	42

Results of observations on acidity relations in the different plantings at given stages of development and of individual plantings at various stages of development are shown in Table XII. For the leaf samples the three topmost leaves, exclusive of the embryonic leaves of the bud, were used. A 2-inch section of the stem immediately below the apical bud and a similar section immediately above the ground level were used. The data are consistent and establish several points of interest. Under the seasonal range in length of day at Washington the response of this sunflower closely coincides with that of cosmos and *Tithonia* when these are exposed to short days. Exactly the same relationship holds with respect to acidity of the cell sap. In the first section of Table XII data are given for seven different plantings of sunflower at 2-week intervals, and it is apparent that the seasonal difference in length of day does not materially affect the acidity of the plant during the first 3 weeks of growth.

Throughout this period the acidity of the tissues in the region of the apex is decidedly less than that of the lower portion of the stem (fig. 8). In all plantings flower buds could be seen about 30 days after germination. The data in the second section of the table show the changes in acidity which are associated with the appearance and development of the flower bud. In earlier stages of development acidity relations in the young, topmost leaves, the upper portion of the stem, and the growing point are much the same. The appearance of the flower bud, however, is marked by considerable increase in acidity in the upper portions of the plant and the flower bud increases progressively in acidity as growth proceeds (fig. 8). Additional observations on other field plantings show further increase in acidity till a  $P_H$  value as low as 5.22 is reached in the fully expanded blossom. On the other hand, as the seeds develop these show decreasing acidity till in large well-filled seeds the  $P_H$  value rises to approximately 7.05.

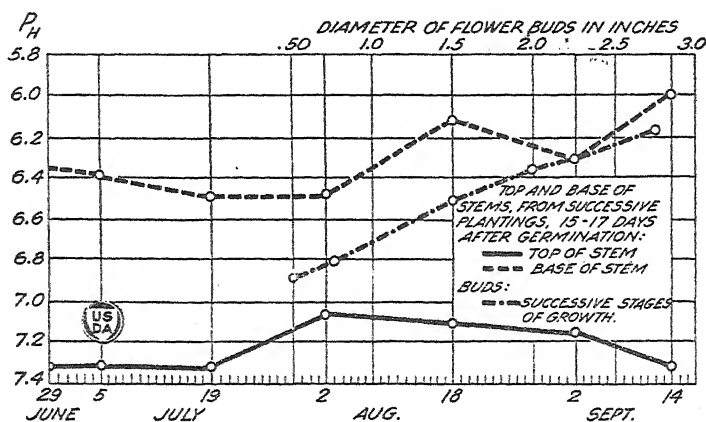


FIG. 8.—Showing the effect of the natural change in length of day of summer and early fall on the active acidity of the top and base of the axis in sunflower and the change in acidity during the development of the flower bud (Table XII). Sunflower resembles the typical long-day plants in that within the seasonal range in length of day (at Washington) the active acidity at the top of the stem is less than that of the base. The flower bud increases sharply and regularly as growth proceeds.

TABLE XII.—Showing the  $P_H$  value of the cell sap of *Helianthus annuus* planted at intervals of two weeks during the summer and at different stages of development

Date of germination.	Date of sample.	Number of days after germination when sampled.	Hour of sampling.	$P_H$ value of sap.					Diameter of flower bud.
				Top-most leaves.	Top of stem.	Base of stem.	Apical bud.	Flower bud.	
June 29.....	July 12.....	13	11.30 a. m.....	7.16	7.33	6.37	.....	.....	inches.
July 5.....	July 22.....	17	11.35 a. m.....	7.25	7.32	6.39	.....	.....	.....
July 19.....	Aug. 3.....	15	12.30 p. m.....	7.14	7.33	6.50	.....	.....	.....
Aug. 2.....	Aug. 19.....	17	1.30 p. m.....	7.29	7.06	6.49	.....	.....	.....
Aug. 18.....	Sept. 2.....	15	1 p. m.....	7.05	7.11	6.12	.....	.....	.....
Sept. 2.....	Sept. 17.....	15	12.35 p. m.....	7.15	7.15	6.31	.....	.....	.....
Sept. 14.....	Sept. 29.....	15	9.50 a. m.....	7.12	7.32	5.99	.....	.....	.....
Aug. 18.....	Sept. 2.....	15	1 p. m.....	7.05	7.11	6.12	.....	.....	.....
Do.....	Sept. 9.....	22	9.50 a. m.....	.....	.....	.....	7.11	.....	.....
Do.....	Sept. 13.....	26	2.50 p. m.....	.....	.....	.....	7.18	.....	.....
Do.....	Sept. 15.....	28	9.20 a. m.....	.....	7.17	6.15	7.11	.....	.....
Do.....	Sept. 23.....	36	9.50 a. m.....	.....	.....	.....	.....	6.89	1 1/2
Aug. 2.....	Sept. 9.....	38	9.50 a. m.....	.....	.....	.....	.....	6.81	3/4
Do.....	Sept. 13.....	42	1.15 p. m.....	.....	.....	.....	.....	6.52	1 1/2
Do.....	Sept. 14.....	43	9.50 a. m.....	.....	6.83	.....	.....	6.36	2
Do.....	Sept. 30.....	59	2.30 p. m.....	.....	.....	6.13	.....	6.17	2 1/2



Observations on plantings which germinated in the greenhouse on January 23 and were exposed to the natural daylight period and to 18 hours of daily illumination, respectively, are recorded in Table XIII. The lengthened illumination period was maintained by use of 100-watt lamps from sunset till midnight, the lamps being placed about 1 foot above the plants. First flower buds were showing on the plants exposed to the natural light period on March 1, or 37 days after germination, and under the 18-hour period first flower buds were seen March 22, or 58 days after germination. For all observations prior to April 6, however, only plants which had not formed flower buds were used, except that one of the four individuals in the short-day series used on March 14 contained a very small bud. It is evident that 18 hours of light daily, as compared with the natural daylight period ranging from about 9½ to 12½ hours, materially delayed the time of flowering and increased the height of the stem. It is clear, also, that the longer light period maintained a higher level of acidity in the upper portions of the plant than did the shorter light period (fig. 9). There is, therefore, a tendency for the sunflower when exposed to an 18-hour light period to show the growth and acidity relations of typical short-day plants like cosmos and Tithonia when exposed to long days. The data in Tables XII and XIII, taken together, show that *Helianthus annuus* forms a connecting link between short-day and long-day plants, since it shows some of the characteristics of both types.

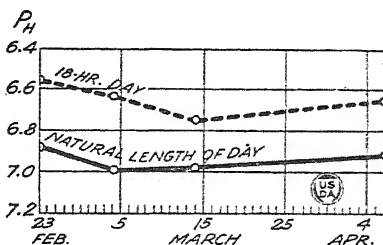


FIG. 9.—Showing the increase in level of active acidity of the apical structures in sunflower when the daily light period is increased to 18 hours. (Table XIII.) Under these conditions flowering is delayed, and in this respect as well as in the increased active acidity of the apex sunflower approaches the behavior of typical short-day plants such as cosmos. Thus, sunflower may be regarded as a connecting link between the more typical short-day plants on the one hand and the typical long-day plants on the other.

TABLE XIII.—Showing the  $P_H$  value of cell sap of *Helianthus annuus* exposed to the natural length of day during the winter and early spring and to 18 hours of daily illumination.

PLANTS EXPOSED TO THE NATURAL LENGTH OF DAY.

Date.	Height.	Hour of sampling.			$P_H$ values.		
		Apical bud.	Top of stem.	Base of stem.	Apical bud.	Top of stem.	Base of stem.
Feb. 23.....	inches 11½	.....	10.50 a. m.	1.55 p. m.	.....	6.88	5.99
Mar. 4.....	17½	10.50 a. m.	..... do. ....	2.55 p. m.	7.04	6.99	6.09
Mar. 14.....	24½	10.55 a. m.	1.15 p. m.	3.20 p. m.	6.81	6.98	6.13
Apr. 6.....	56½	10.45 a. m.	1.30 p. m.	4 p. m.	6.44	6.91	6.18

PLANTS EXPOSED TO 18 HOURS OF ILLUMINATION DAILY.

Date.	Height.	Apical bud.	Top of stem.	Base of stem.	$P_H$ values.		
Feb. 23.....	inches 20	.....	10.10 a. m.	12.55 p. m.	.....	6.56	5.78
Mar. 4.....	31¼	9.50 a. m.	1.05 p. m.	2.55 p. m.	6.82	6.63	6.10
Mar. 14.....	45½	9.55 a. m.	12.15 p. m.	2.20 p. m.	6.73	6.76	6.16
Apr. 6.....	86¼	9.45 a. m.	12.35 p. m.	2.50 p. m.	6.50	6.65	6.17

• Young flower buds, 1 to 1½ inches in diameter.

## ACIDITY RELATIONS DURING THE PERIOD OF TRANSITION FROM THE VEGETATIVE TO THE FLOWERING CONDITION

The data which have been presented in the preceding paragraphs show that decided change in the hydrogen-ion concentration of the cell sap is closely associated with change in the course of development of the plants as induced by increasing or decreasing the duration of the daily illumination period. Thus, change from long to short days, which promptly initiates flowering in such plants as cosmos and Biloxi soy beans, also results in marked change in the active acidity of the plant sap. The question arises at once as to the possible significance of the change in acidity. In this connection the time and extent of the change in acidity in relation to the time at which actual transition from one type of activity to another

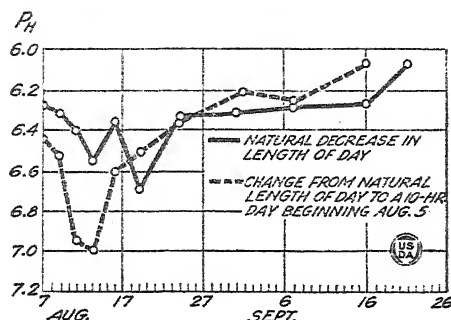


FIG. 10.—Showing the temporary, sharp decrease in active acidity of the cell sap in Biloxi soy beans which results from suitable decrease in the daily illumination period (Table V). This change in active acidity apparently marks the transition from the vegetative to the reproduction stage. The abrupt shortening of the daylight period produces a more decisive effect both in the temporary fall and in the subsequent rise in active acidity. The gradual, natural decrease in length of day seems to cause a less decisive wavering effect, probably indicating a rather delicate balance between the alternative vegetative and reproductive forms of activity.

to approximately the original level. This is followed by a further, slow increase during the development of the reproductive organs.

These changes in acidity in Biloxi soy beans, as recorded in Table V (p. 137), are brought out more clearly in figure 10. It is believed that the sharp decline in acidity of the sap, occurring in this case 5 to 8 days after transfer to the 10-hour day, definitely marks the actual transition from the vegetative to the flowering stage. It will be observed that a similar but somewhat less pronounced decrease in acidity occurred a few days later in the plants exposed to the natural decrease in length of day. These plants began flowering September 15 and it is known that after suitable change in length of day has occurred a minimum period of about 25 days must elapse before the appearance of open blossoms. Hence, initiation of flower-bud formation must have been induced at about the time of the sharp decline in acidity. Since the natural decrease in length of day is gradual, it is to be expected that a critical point must occur at which there will be a condition of unstable equilibrium. A glance at figure 10 will disclose distinct evidence of such a wavering or "wobbling" action, the final transition from vegetative to flowering condition apparently occurring about August 19, just 27 days before the appearance of open blossoms.

takes place is of special interest. It is obvious that abrupt change from long to short days acts very quickly in initiating the formation of flower primordia in typical short-day plants, so that any causal factor must be operative within a few days after the change in light exposure occurs. It has already been pointed out that decided change in acidity usually becomes apparent about three to five days after the change in light conditions has been made. At this time there is a sudden and marked decrease in acidity. This decrease, however, is only temporary and is promptly followed by abrupt increase in acidity

In the preceding experiment the soy beans were not planted till July 1 and consequently under the natural length of day flowering began about 10 days later than the normal date of flowering for earlier plantings. In repeating the experiment, the results of which are shown in Table VI (p. 138), plantings were made May 15, and in this case flowering began September 4 under the natural length of day. The changes in acidity as shown in Table VI are much the same as in the previous experiment, under both the natural length of day and the 10-hour day. In this instance flowering apparently was initiated about July 27, but, because of the greater length of the day as compared with that prevailing at the transition period in the preceding experiment, 39 days were required for the appearance of open blossoms. This is in agreement with previous experience (see 8, p. 882).

There is no satisfactory evidence that the drop in acidity of the sap which occurs during the transition from the vegetative to the flowering stage stands in a causal relation to this phenomenon. It seems more likely that the sudden decrease in acidity marks a temporary cessation of metabolic activity of the type concerned in stem elongation and of which the acidity itself is a product. It is to be remembered that light is not necessary for the decomposition of organic acids in the plant, while, on the other hand, exposure to light does result in the formation of these acids. The above results, if they have been correctly interpreted, are of interest, however, in indicating the triggerlike character of the transition from the vegetative to the flowering condition and furnishing a means of recognizing the very early stages in the series of transformations involved. It is quite possible, moreover, that these changes in acidity, as a link in the chain of events set up, in the first instance, by change in the light period, have important end results in influencing plant metabolism. The acidity relations in the plants under the 10-hour day in comparison with those under the natural length of day, as brought out in figure 10 and Tables V and VI, are in full agreement with the observed responses of the plants under the two conditions. Similar relationships are seen, also, in the data obtained with cosmos, *Tithonia*, and other species.

#### CARBOHYDRATE RELATIONS IN PHOTOPERIODISM

In view of the attention which plant physiologists have given in recent years to carbohydrate content of the plant as a factor in flowering and fruiting it seems desirable to include in the present paper the results of preliminary observations on the influence of length of day on the concentration of soluble carbohydrate in the tissues of the plant. Discussion of the literature on the general subject of carbohydrate content in relation to sexual reproduction is deferred till more complete data are available on effect of duration of the light period on the carbohydrate relations of the plant. In this connection mention should be made of recent work by Nightingale (21) dealing with the effect of the light period on the behavior and the chemical composition of certain plants. This author suggests that the duration of the light period is a factor in the utilization of carbohydrate for the conversion of nitrate nitrogen into protein form. In certain varieties of buckwheat, soy beans, radish, and salvia a 7-hour day limited the synthesis of nitrates to insoluble forms of nitrogen, even though an available supply of carbohydrate was present. In tomato plants already containing an abundance of carbohydrates shortening the light period caused marked decrease of carbohydrate

content coupled with decomposition of insoluble nitrogen. Under these conditions there was new growth, although there was no external supply of nitrates. As bearing on these relationships it may be pointed out that the relative capacity to effectively utilize carbohydrate for promotion of growth, the physiological translocation of plastic nutrients upward or downward in the plant, and the "reworking" of nitrogen and other plant-food elements present in limited quantities as a result of appropriate change in the light period have been discussed in a previous paper (8, pp. 896, 907) in so far as these phenomena are made evident by the observed responses of the plants.

Biloxi soy beans were planted June 29 in the field and samples of leaves were collected at intervals of two to three weeks for determination of soluble carbohydrates and nitrogen in the cell sap. The plants began flowering September 12. The leaf samples consisted of sets of three upper full-size leaves of the plant. The samples were collected at 10.30 a. m. The material was frozen and the sap expressed and filtered. To preserve the sap for sugar determinations, alcohol was added in quantity to form a 60 per cent solution after mixing, while for preserving the samples for nitrogen determination sulphuric acid was added to form a 4.5 per cent solution after mixing. Reducing sugars were determined, before and after inversion with hydrochloric acid, according to the methods for foods and feeding stuffs of the Association of Official Agricultural Chemists. The nitrogen determinations were made according to the official Kjeldahl method modified to include nitrogen in nitrates. The results are shown in Table XIV. So far as may be judged by these data there is a slight increase in reducing sugar in the leaf at about the time flower buds are laid down, followed by a decrease during the period of unfolding the first blossoms. Three weeks after the flowering stage is reached, however, when seed development is well under way, there is a marked increase in reducing sugar and a small increase in soluble nitrogen. There appears to be little if any polysaccharid present in the sap at any stage. The data are not sufficient to justify final conclusion, but as far as they go seem to indicate that the most marked accumulation of soluble forms of carbohydrate and nitrogen in the leaf takes place after flowering has been initiated and during the active development of the fruit.

TABLE XIV.—Sugars and nitrogen contained in 10 cc. portions of sap of Biloxi soy beans taken at intervals during the growing season.

Date of sample.	Reducing sugars.			Nitrogen.
	Before inversion. (a)	After inversion. (b)	Difference. (b-a)	
	Gm.	Gm.	Gm.	Gm.
July 26.....	0.0934	0.0954	0.002	0.0219
Aug. 16.....	.1230	.1060	.003	.0247
Sept. 7.....	.0750	.0786	.0036	.0226
Sept. 21.....	.0764	.0766	.....	.0236
Oct. 6.....	.1692	.1764	.0072	.0290

Late-flowering *Cosmos bipinnatus* was planted in boxes out of doors on May 10. On August 5, when the plants were about 60 inches high, one lot was placed under a 10-hour day. Under the 10-hour day flower

buds were first seen on August 17 and under the natural length of day they were observed on September 21. Samples for analysis, consisting of upper sections of the stems 18 inches in length, were collected at intervals, the attached leaves being removed and rejected. The samples were taken at 1 to 2 p. m. After taking the green weights of the stems, these were cut into 2-inch pieces and plunged into boiling alcohol to which a small quantity of calcium carbonate had been added. Boiling was continued for 15 minutes. Extraction of sugars from the stem material was completed with 50 per cent alcohol. Reducing sugar was determined before and after inversion. The water content of the samples was obtained by drying the extracts and residues at 100° C. and deducting the weights from the green weights of the samples. The results of the analyses are given in Table XV. There is in these data some indication of change in water content of the tissues as a result of change in the light period. Apparently a slight decrease in water content had taken place four days after the transfer to a 10-hour day had been made, while 12 days later, after flower buds had appeared, the water content had risen to 2 per cent above that of the plants exposed to the natural length of day. In this connection it is to be noted that this increase in water content occurs at a time when exceedingly rapid elongation of the flower stems takes place.

TABLE XV.—*Water and sugars contained in the upper portion of the stem of late-flowering cosmos as affected by transfer August 5 from the natural length of day of summer to a 10-hour day.*

Date.	Water content.		Total reducing sugar after inversion.		Reducing sugar before inversion.		Sucrose.	
	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.	Natural length of day.	10-hour day.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
August 7 .....	84.95	85.18	18.41	21.02	8.15	10.40	9.75	10.09
August 9 .....	85.00	84.64	14.02	20.80	6.37	6.85	7.27	13.25
August 21 .....	84.89	86.74	16.25	20.75	7.79	10.79	8.04	9.46

It is plain that shortening the light period had a prompt and decided effect on the content of soluble carbohydrate. Two days after the change had been made there had been an increase amounting to 14 per cent of total sugars originally present. Thereafter, total sugars remained practically constant under the 10-hour day, although there was considerable fluctuation under the longer length of day. It will be observed that transfer to the short-day conditions affected the relative percentages of monosaccharid and polysaccharid. The increase of carbohydrate within the first 48 hours consisted almost entirely of reducing sugar, while 2 days later, with the total sugar content remaining constant, there was a marked increase in polysaccharid at the expense of the reducing sugar originally present. This change in proportion of monosaccharid to polysaccharid seems to be temporary, and subsequently the tendency is toward a return to a more nearly equal distribution between the two classes of sugars. Further studies are under way to determine whether these relationships are characteristic features of the effect of the light period on internal conditions of the plant in association with

change from the vegetative to the flowering condition induced by the light factor. There is no apparent reason for supposing that these changes in carbohydrate content are directly connected with photosynthesis, since the increase in soluble carbohydrate is caused by decrease in the duration of the illumination period.

Scarlet Globe radish was planted in the greenhouse September 29, and one lot of plants was allowed to grow under the natural length of day while a second lot was exposed to an 18-hour day, using electric light to prolong the daylight period. On January 10 the two lots of plants were sampled for determination of reducing sugar in the sap. Under the short day the plants sampled had not developed flowering stems but under the long day the plants had developed stems 20-24 inches high and these were showing flower buds at the tips. In sampling the thickened root only a central horizontal section was used. In the leaf material only the stem was used, the lamina being discarded. The stems were divided into upper and lower portions, the point of division being somewhat below the center. Leaf samples were taken at 10.30 a. m. and the other material at 1 p. m. The saps were prepared by grinding and pressing the material without freezing and then filtering the expressed juice. The results of the analyses are shown in Table XVI. In brief, it seems that under the longer light period the content of reducing sugar is considerably greater than under the relatively short light period and the concentration in the upper portion of the stem is much greater than in the lower. Nightingale (21) also found rapid upward translocation of carbohydrate in radish exposed to a long day. Apparently the increase in reducing sugar under the longer illumination period is not due simply to increased photosynthesis, since under these conditions the thickened root undergoes shrinkage as a result of dissolution and translocation of storage forms of carbohydrate.

TABLE XVI.—*Reducing sugar in sap of radish exposed to an 18-hour day and to the natural length of day of winter.*

Material.	Reducing sugar in 25 cc. of sap, calculated as dextrose.	
	Plants exposed to an 18-hour day.	Plants exposed to the natural length of day.
	Gm.	Gm.
Upper portion of primary stem.....	0.1192	.....
Lower portion of primary stem.....	.0185	.....
Leaf stem.....	.0332	0.0185
Middle section of tuber.....	.0292	.0193

### CONCLUSIONS

The phenomena of photoperiodism as presented in this and preceding papers emphasize the fact that in addition to influencing the fundamental process of photosynthesis—that is, conversion of the chemical elements of carbon dioxide and water into carbohydrate—the duration of the daily light period may definitely control other parallel processes of fundamental importance in plant growth and development. The light period may determine not only the quantity of carbohydrate produced but also the utilization of this material, and it is not possible to explain either the

definite formative effects of the light period or its action on the growth rate on the basis of photosynthesis alone. What these additional processes are has not been determined, but there can be no question as to their fundamental importance. In the present paper data are presented which indicate that the light period in some way profoundly influences acidity relations, the form of the carbohydrate present in the plant and probably the water content of the tissues. Daily periodicity in content of total uncombined acid as affected by light is an outstanding feature of acidity relations in fleshy plants, but in the types of thin-leaved species here dealt with this daily periodicity is of much smaller magnitude. In the present paper the influence of the relative length of day and night on the average level of active acidity in the plant, as measured by the hydrogen-ion concentration of the sap, is considered in some detail. It is shown that growth relations and definite form of expression as controlled by length of day are regularly associated with characteristic acidity relations.

In the case of short-day plants, indeterminate upward elongation of the vegetative stem, which is a characteristic response to a relatively long daily illumination period, is associated with progressive increase in active acidity of the plant, particularly in the region of the growing point. This increase continues till the upper portions of the plant become more acid than the lower portions. On the other hand, exposure to a relatively short daily light period sharply limits increase in stature and quickly initiates flowering and fruiting. Under these conditions a brief transitory period of decreased acidity is followed by only a moderate increase until a level is approached at which flowering is initiated. This level of acidity is much below that characteristic of the advanced vegetative stage under long-day conditions. Under the short-day exposure the upper portions of the plant are less acid than the lower portions. After flowering has been initiated there is progressive increase in acidity in the vegetative parts of the plant. As to the reproductive structures themselves, the embryonic flower bud is relatively low in acidity, while growth of the bud is accompanied by increasing acidity which reaches a maximum in the unfolded blossom. The developing seed, on the other hand, shows a progressive decline in acidity during the period of active growth. Abrupt transfer from a long day to a short day causes a sudden and sharp decrease in acidity in the region of the growing point, which usually occurs about three to five days after the transfer has been made. This drop in acidity, which is believed to indicate definite transition from the vegetative to the flowering condition, is only temporary and is followed by an equally rapid rise to approximately the original level of acidity. These changes in acidity are also observed when flowering is initiated by natural decrease in length of day, but the extent and the sharpness of the changes in acidity are more or less proportional to the amount of the change in duration of the light period. The acidity relations resulting from exposure to the long days of summer also obtain when the short daylight period of winter is prolonged by use of electric light of low intensity.

In the case of long-day plants exposure to a relatively short day tends to inhibit stem elongation, resulting in the leaf-rosette type of development, with or without tuberization. Under these conditions the acidity of the plant remains at a relatively low level. Exposure to a relatively long day more commonly results in elongation of the axis, followed by

flowering. This form of development is associated with general increase in acidity. There appear to be some differences in detail, however, as to the acidity relations in the type of plant represented by summer radish, in which tuberization is a prominent feature, as compared with the type represented by *Rudbeckia bicolor*, in which tuberization ordinarily does not occur. In radish, increase in the duration of the daily light period causes increased acidity in the leaf, and acidity is greater in the upper portion of the stem than in the lower even in the earlier stages of stem elongation. In *Rudbeckia*, increase in the light period causes decreased acidity in the leaf, and the upper portion of the stem is consistently less acid than the lower portion until after the blossom has unfolded. In both radish and *Rudbeckia*, however, the acidity of the upper portion of the stem increases progressively both before and after the appearance of flower buds. The acidity relations in the *Rudbeckia* type of plant when exposed to long days are strikingly similar to those of short-day plants when the latter are exposed to short days. In both instances the acidity of the stem increases moderately prior to the appearance of flower buds, and the reproductive structures themselves increase in acidity as growth proceeds, maximum acidity being found in the unfolded blossom. Moreover, the upper portion of the developing stem is less acid than the lower portion, in contrast with conditions in short-day plants when these are exposed to long days.

There is a group of plants which occupy a position intermediate between the more typical short-day and long-day groups in their responses to differences in the length of day. This group shows some of the features of both short-day and long-day plants. Thus, in *Helianthus annuus* the time of flowering is not materially influenced by the natural range in length of day of spring and summer at Washington, but the stature attained is greatly affected. Increasing the daily light period to 18 hours, moreover, causes considerable delay in flowering. Thus, under the relatively long days of summer this species shows much the same behavior as do short-day plants when they are exposed to short days or long-day plants when exposed to long days. It was found also that the same relationships hold as to acidity of the cell sap. On the other hand, when exposed to an 18-hour illumination period this sunflower approaches in behavior that of short-day plants when exposed to long days. Here, again, the rise in acidity of the sap resulting from the longer light period shows an approach toward the condition of high acidity found in the short-day plants when exposed to long days. This type of plant, therefore, forms a connecting link between the two groups of short-day and long-day plants.

Preliminary studies, which are now being followed up with more extensive observations, indicate that changes in form of the carbohydrate content and in the degree of hydration of the tissues of the plant are among the earliest observable effects of change in the length of day to which the plant is exposed. Transfer of cosmos plants from a long to a short day resulted in a material increase in reducing sugar in the upper portion of the stem within 48 hours after the transfer had been made. Two days later the increase in sugar content was found to be in the form of polysaccharid, and apparently this was accompanied by a slight decrease in water content of the tissues. Twelve days later, after flower buds had appeared, the increased content of sugar was again in the form of monosaccharid. A notable increase in water content of the tissues also had taken place. In Biloxi soy beans exposed to natural length of



day of late summer and fall a slight increase in reducing sugar was found in the sap of the leaf at about the time flower buds were laid down, followed by a decrease at the time open blossoms appeared. A marked increase in reducing sugar and a smaller increase in soluble nitrogen was observed 15 days later, when development of the fruit was rapidly progressing. In summer radish, elongation of the stem resulting from exposure to a long day is associated with an increased content of reducing sugar in the tissues and maximum concentration is found in the upper portion of the stem.

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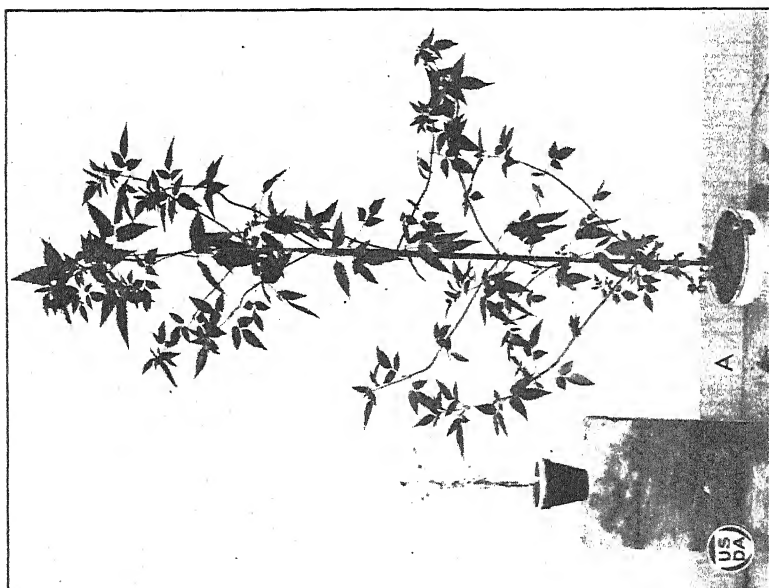
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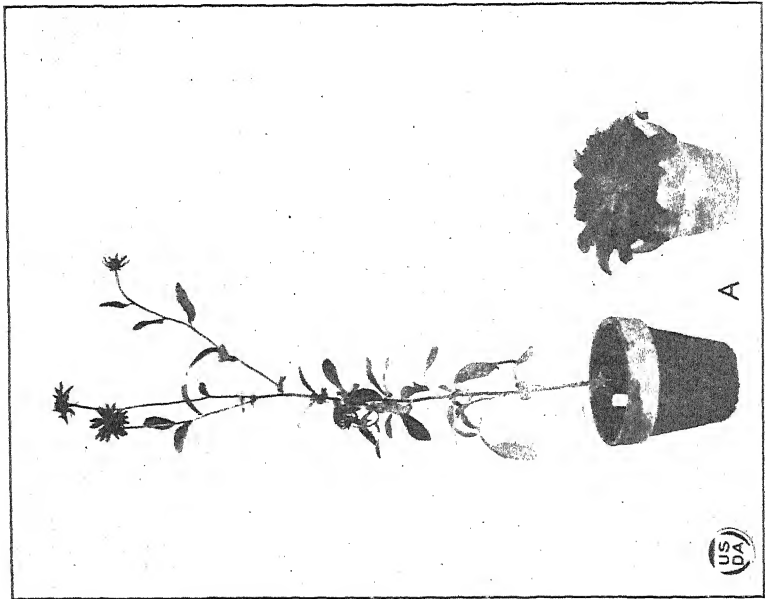
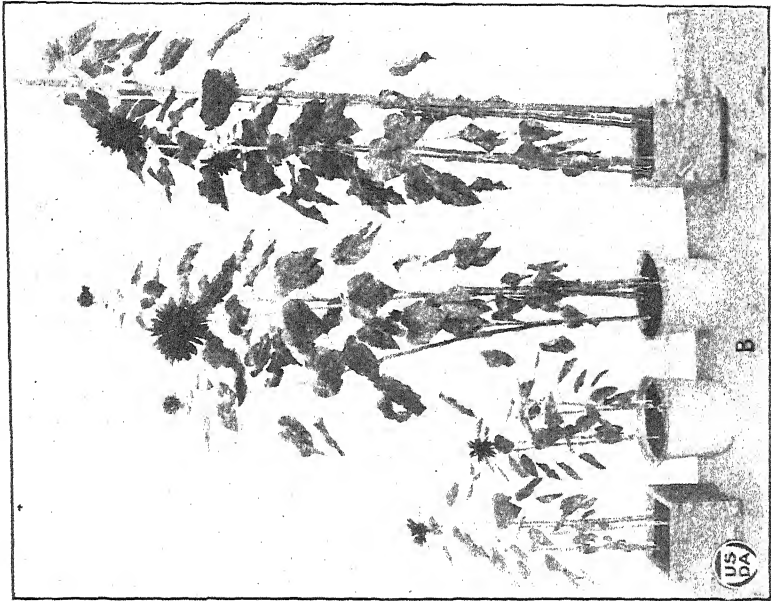


## PLATE 1

A.—*Bidens frondosa* L. Plants from seed which germinated December 1. The individual at right was exposed to the natural daylight period of winter supplemented with electric illumination of low intensity from sunset till midnight each day. Individual at left received similar treatment till January 30 after which it was exposed to the natural daylight period only. Shortening the duration of the daily illumination period promptly initiated flowering and open blossoms were seen March 1, the plant having attained a height of 15 inches. When the photograph was made, April 17, the plant at left had ripened its seed and was dead. The plant at right, which remained under the long daily illumination, had attained a height of 6 feet without flowering. This species shows the typical behavior of short-day plants.

B.—Late-flowering cosmos (*C. bipinnatus* Cav.), showing change from the flowering condition back to the vegetative stage as a result of increase in length of the daylight period to which the plant was exposed. This plant, which germinated May 15, was exposed to a 10-hour day till June 13, and thereafter it was exposed to the full daylight period of summer. Flower buds appeared as early as June 3 and flowering had begun by June 19. It is apparent that when photographed, July 20, the new branches developing under long-day conditions were primarily of the vegetative type, though some flower buds were still developing. Though the indeterminate vegetative type of growth is thus restored by increase in length of the daily illumination period, the flowering condition, once it has been established, is completely destroyed only very slowly and with difficulty, if at all.





## PLATE 2

A.—*Rudbeckia bicolor* Nutt. Plants from seed sowed December 19. The individual at left was exposed to the natural daylight period of winter supplemented by electric illumination from sunset till midnight. The individual at right received only seven hours of illumination daily. Photographed May 14. The behavior of *Rudbeckia* is typical of the group of plants which quickly flower in response to relatively long days, and therefore are designated as long-day plants. Ordinarily in this group the height attained is more or less directly proportional to the length of day.

B.—*Helianthus annuus* L. Seedlings which germinated January 23. The larger plants at right were exposed to the natural daylight period of winter and spring, while the smaller plants at left received only seven hours of illumination daily. Photographed April 12. The time of flowering was not affected by the reduced illumination period, but the height attained was markedly decreased. On the other hand, if the illumination period is increased to 18 hours, the time of flowering is delayed. This sunflower shows some of the characteristics of both short-day and long-day plants.





# ON THE ANATOMY OF THE SWEET POTATO ROOT, WITH NOTES ON INTERNAL BREAKDOWN

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## INTRODUCTION

The fleshy roots of the different varieties of *Ipomoea batatas* Lam. are fusiform, napiform, or irregular-spherical. The surface of the skin appears uniform and smooth in some varieties, and in others irregularly ribbed, because of the presence of vein-like prominences. The secondary roots are arranged in more or less straight vertical rows. Each of these rootlets sits in a shallow depression surrounded by an arch of rough, scar-like tissue, which in shape and texture closely resembles a potato eye. Occasionally the fleshy roots are deeply lobed so that the rows of lateral rootlets lie in longitudinal grooves.

The root nature of these fleshy structures was first shown by Turpin (6),<sup>2</sup> who published figures comparing the roots of *Ipomoea* to the tubers of the Irish potato and the Jerusalem artichoke. Recently Kamerling (3) and Tuyihusa (7) have taken exception to this view and claimed that these fleshy structures are modified stems. But the validity of this assumption, however ably defended otherwise, becomes untenable when young material is studied. The exarch position of the protoxylem decides, without further argument, in favor of the root-structure theory of these organs.

Since the sweet potato belongs to a group of plants which are distinguished by a peculiar anomalous growth, its anatomical structure has been studied indirectly by a number of investigators, notably by Schmitz (5). The only direct contribution to the knowledge of the internal structure of the sweet potato is a short treatise by Miss McCormick (4), who also has reviewed all pertinent literature.

## THE STRUCTURE OF THE YOUNG ROOT

The young root shows in transverse section several groups of vascular tissue, separated from a thick cortex by an endodermis. (Fig. 1.) The vascular tissue is arranged radially, the xylem and phloem in alternating strands. The number of protoxylem points varies, but most of the roots are pentarch or hexarch. In the region near the growing point, the first protoxylem elements to mature are those farthest away from the center; the development therefore is centripetal. The later maturing protoxylem elements approach each other more and more closely with the increasing age of the region. The protophloem forms small, oval groups lying between the strands of protoxylem and within the circle of the youngest protoxylem element. A parenchymatous sheath, composed of small cells which lack intercellular spaces, intervenes between phloem and

<sup>1</sup> Accepted for publication Nov. 24, 1923.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 166.

xylem. Both xylem and phloem are separated from the endodermis by a single-layered pericycle.

The cortex forms a broad band of tissue composed of large cells with conspicuous intercellular spaces. The peripheral cells are covered by a root epidermis which soon becomes torn and is later replaced by a periderm.

The secondary rootlets take their origin in the pericycle from meristematic cells opposite the protoxylem groups. There are commonly as

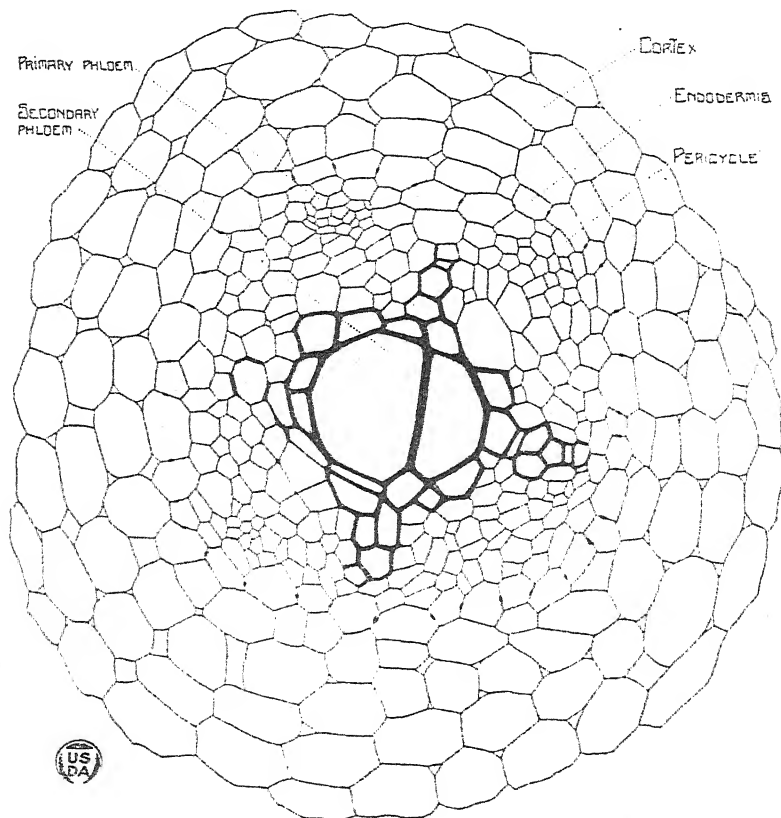


FIG. 1.—Cross section of a young rootlet.

many rows of lateral rootlets as there are protoxylem points. This correlation is well marked in certain overgrown roots, which show five or six prominent ridges and the same number of rows of lateral rootlets in the intervening grooves.

#### EARLY DIFFERENTIATION AND DEVELOPMENT OF THE ROOT

Even before the protoxylem points are differentiated sufficiently to effect union in the center, growth activity becomes evident in the central parenchyma. Due to this cell increase, the protoxylem groups are forced outward. Meanwhile, one of the most centrally located parenchyma cells has greatly enlarged and matured into a large xylem element.

(Pl. 1 A and fig. 3.) Occasionally an additional small vessel is formed adjacent to the large one.

Following the differentiation of this large "central cell" and the formation of an area of actively growing tissue between the central cell and

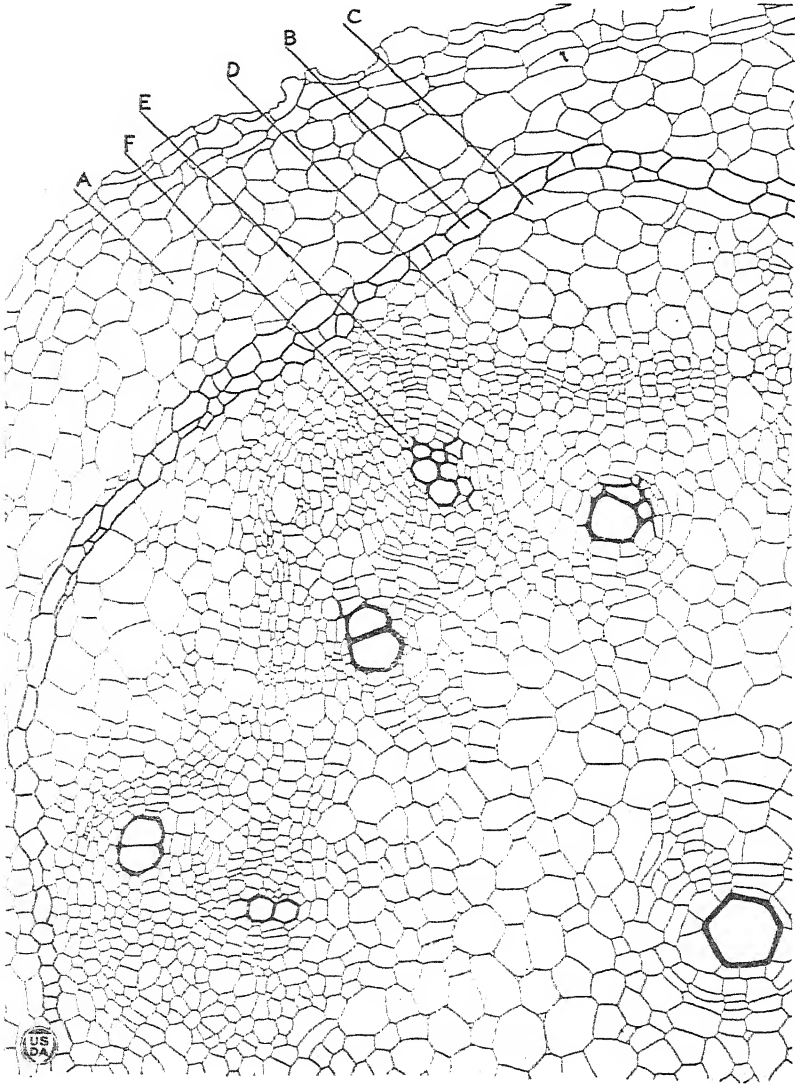


FIG. 2.—Cross section of a young fleshy root. A, cortex; B, endodermis; C, pericycle; D, phloem; E, cambium; F, proto-xylem group. Drawing is a copy of an enlarged photomicrograph.

the protoxylem groups, the differentiation of a cambium takes place. This cambium, lying outside of the xylem and inside of the phloem, gradually forms a complete cylinder. The outline of this cylinder is at first very irregular (fig. 3), partly because of the radial arrangement of the vascular tissue and partly because of local cell increase in the

pericycle opposite the phloem groups. As the root enlarges, cells are produced by the cambium more rapidly in the inner region, and the cambium cylinder becomes symmetrical. Occasionally the irregularities in the activity of the cambium ring continue to be present throughout the growth of the tuber, causing the formation of deep invaginations which are especially characteristic of certain varieties, as Nancy Hall and Belmont.

The endodermis of the young root is very prominent, but gradually the cells become stretched in the tangential plane and show signs of disorganization. The cells of the cortex increase in number to accommodate the widening circumference of the growing organ. The intercellular spaces grow larger, whereby the tissue acquires a very loose texture; the epidermal cells become torn and lignified.

Those parts of the sweet potato roots which do not become thickened undergo similar changes in their ontogeny. But early during the differentiation processes, the cells between the protoxylem points and the large central cell become lignified (fig. 1). Miss McCormick (4) states that a certain amount of xylem is developed in all young roots; but when a root enters upon tuber formation, the xylem mass is broken up by the development of a parenchymatous sheath between its elements. This observation, however, was not verified. The enlargement of the young roots into fleshy roots is initiated at a very early stage in the development of the root, and always precedes the differentiation of xylem on the inner face of the protoxylem groups.

With the differentiation of the cambium cylinder, the cells around the protoxylem groups begin to show a more regular arrangement. Soon a cambium becomes distinct around each of the groups. This development of secondary cambiums is not limited to the protoxylem groups, but becomes a general phenomenon. (Fig. 6). Simultaneously with the formation of secondary cambiums around the protoxylem groups, a new meristematic zone develops around the central cell. At first only one cell wide, the number of cell rows of this new cambium increases rapidly and soon forms a tangential band of appreciable width. At a somewhat later stage a number of cells in close proximity to the primary cambium mature into xylem. They, in turn, become surrounded by a secondary cambium which continues activity for a limited period. In the later development of the root there appear, independent of the vascular groups, cambiums in the form of bands or circles, producing xylem toward the center and phloem toward the periphery. In the original phloem groups new cambiums may also arise, which produce secondary elements in the regular manner.

The primary cambium cylinder is meanwhile actively dividing, forming xylem and phloem, but mostly thin-walled storage parenchyma. The xylem elements are few in number, yet show a certain regular arrangement in radial rows. Around each of the newly formed xylem cells a complete or partial cambium may develop, which produces new elements in the regular manner. Often, when the amount of tissue produced by the secondary cambium is considerable, as can best be seen in the region of the large central cell of the young root (Pl. 1, B), a tertiary cambium develops around a number of the secondary elements and increases in the same way as did the group from which it arose. Sections through fleshy roots, which exhibit in mature condition the typical ridged structure previously mentioned, show the ridges traversed by vascular bundles. (Fig. 4.) These bundles originate in the primary cambium cylinder,

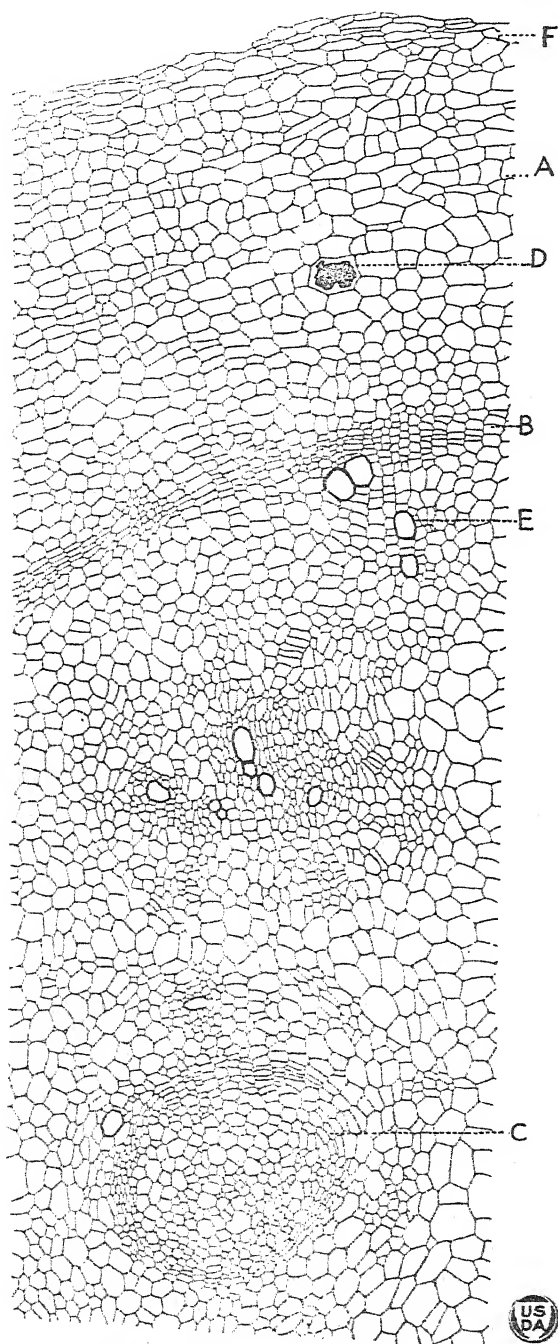


FIG. 3.—Partial cross section of fleshy root about 10 mm. in diameter. A, cortex; B, cambium; C, secondary circular cambium; D, latex cell in cortex; E, secondary xylem, F, periderm.

one another by a single large pore. The lumen of the larger cells becomes filled with tyloses which are most prominent in the mature root.

The storage parenchyma is of two types: (a) Normal bundle parenchyma which, like the xylem and the phloem, is a product of the cambium; (b) interstitial parenchyma—a filler between the groups of bundles. The interstitial parenchyma can be considered the direct progeny of the cells of the parenchymatous sheath of the young rootlet. It forms irregular areas and even broad zones, which are distinguishable from the surrounding tissue by their lighter color. The cells composing it are commonly irregular, elongated, and poor in starch. The cells of the bundle parenchyma are polyhedral and fairly uniform; they are very rich in starch and possess a well developed nucleus.

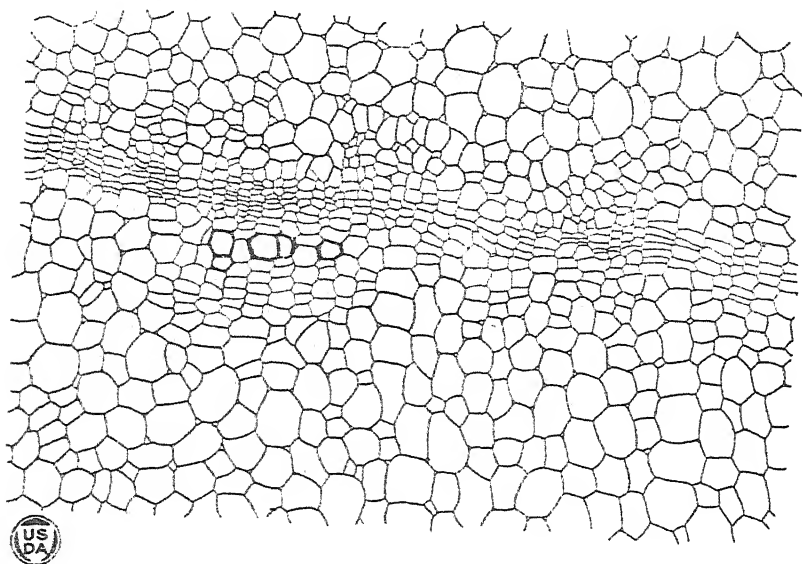


FIG. 6.—Cross section of cambium of large fleshy root. Notice the formation of a secondary cambium to the inside of the group of xylem elements.

#### TISSUE BREAKDOWN IN STORAGE

Harter and his coworkers (2) observed that certain sweet potato roots which had been in storage for a number of weeks would show symptoms of internal breakdown. An examination of the affected areas showed that the cells were partly destroyed and that their starch content was greatly reduced. In general, however, the tissues exhibited no chemical changes which would indicate far-reaching pathological disturbances.

From the study of the structure of the normal tuber, it will be recalled that the groups of vascular tissue are separated by undulating bands or areas of parenchyma. When tubers in the initial stage of breakdown are examined, it becomes evident at once that the first pathological disturbance has taken place in the interstitial parenchyma. The cells of this tissue are large, irregular, and poor in starch; vascular elements and latex tubes are entirely wanting. When breakdown occurs, the cells of the affected region become at first more or less dehydrated; this is indicated by their infiltration with air, giving them a pure white appearance

whereby they stand out strikingly from the surrounding tissue (Pl. 4, A). This parenchyma gradually acquires a spongy texture as the obliteration of cells progresses, leading finally to the formation of small, polyhedral chambers which are lined by the remnants of the destroyed tissue. (Pl. 4, A, B, C, D.)

This process of progressive obliteration begins simultaneously at many points, spreading slowly from the center of the fleshy root along the vertical axis and in extreme cases involving the entire root. During the formation of these cavities, transverse zones of tissue may remain in certain places, forming diaphragms which break the continuity of the longitudinal hollows (Pl. 4, A). The diaphragms appear as thin, translucent membranes from one to several cell layers thick. The walls of the cells are for the most part still cellulose, but some of the elements become lignified. In severely affected fleshy roots the diaphragms also break down, so that long, continuous air passages traverse the entire root.

In advance stages of breakdown, obliteration of the tissue spreads to the parenchyma of the individual bundles, resulting in the denudation and complete isolation of numerous vascular strands.

Except for the lignification of individual cells, the tissue lining the cavities undergoes no change in the composition of its cell walls. Occasionally, however, roots show a pronounced discoloration of the cavities, and a browning and partial lignification of the tissue bordering the hollows. The discoloration may remain more or less confined to the lining of the cavities, or it may spread so as to involve several layers. At such a stage, a section through a root shows a picture not unlike that produced by certain dry rots. The apparent sterility of these cavities and the absence of external injuries, however, suggest no complications resulting from parasitic infections, and the browning of the tissues must be considered, for the present at least, as an advanced stage of the original breakdown.

The hollows which result from the breakdown of the fleshy root tissue resemble in their appearance the lysigenous air spaces found in the stems, leaves, and roots of grasses; sedges, and a host of other plants. The formation of these air passages, according to de Bary (1, p. 216),

begins by those cells which do not follow the growth of the tissue surrounding them, becoming at first separated from one another so as to form schizogenous cavities which gradually increase in size. The cells of the tissue thus broken up then gradually lose their protoplasm, dry up and coalesce as flaky masses which are attached to the wall of the cavity. In other cases, the cells first lose their protoplasm, the membranes become apparently thinner, and finally rupture by the extension of the surrounding tissue.

In all these cases the formation of air cavities is a normal phenomenon in the growth and differentiation of the organs of certain plants. The cavities themselves are partly the result of unequal growth of the tissues. Similar agencies are at work when, under abnormal climatic conditions, organs such as Irish potato tubers show extensive internal hollows. These cavities, however, do not develop after the tuber is mature and placed in storage.

While the cavities occurring in the sweet potato have much in common, as far as appearances go, with those resulting in other plants from normal growth processes, and though the mechanism of their formation might be the same, or similar, if we consider typical lysigenous cavities, we have still to determine what factors are responsible for such changes in the

normal metabolism of the resting root. Since breakdown is, to all appearances, correlated with metabolic changes in the resting root, it might be possible to find a satisfactory explanation on a purely mechanical basis by ascertaining the chemical composition of susceptible and resistant fleshy roots and noting the changes which take place under various conditions of storage. From such evidence it may be possible to determine the origin of the trouble, or at least to discover significant correlations which may point to the solution of the problem.

### SUMMARY

The edible structure of *Ipomoea batatas* is a thickened root. Its peculiar anomalous structure is due partly to the action of a primary cambium, partly to the development of secondary cambiums and their products.

The cells of the interstitial parenchyma of certain of the varieties break down in storage, causing the formation of polyhedral chambers lined with the cottony débris of the disintegrated tissue.

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PLATE I.

A.—Cross section of young, fleshy root (4 mm. diameter). Notice prominent endodermis.

B.—Cross section of large vascular bundle of interior of mature fleshy root. Notice the development of a tertiary cambium around a group of xylem cells in the center of the figure. (Lines are partly redrawn.)



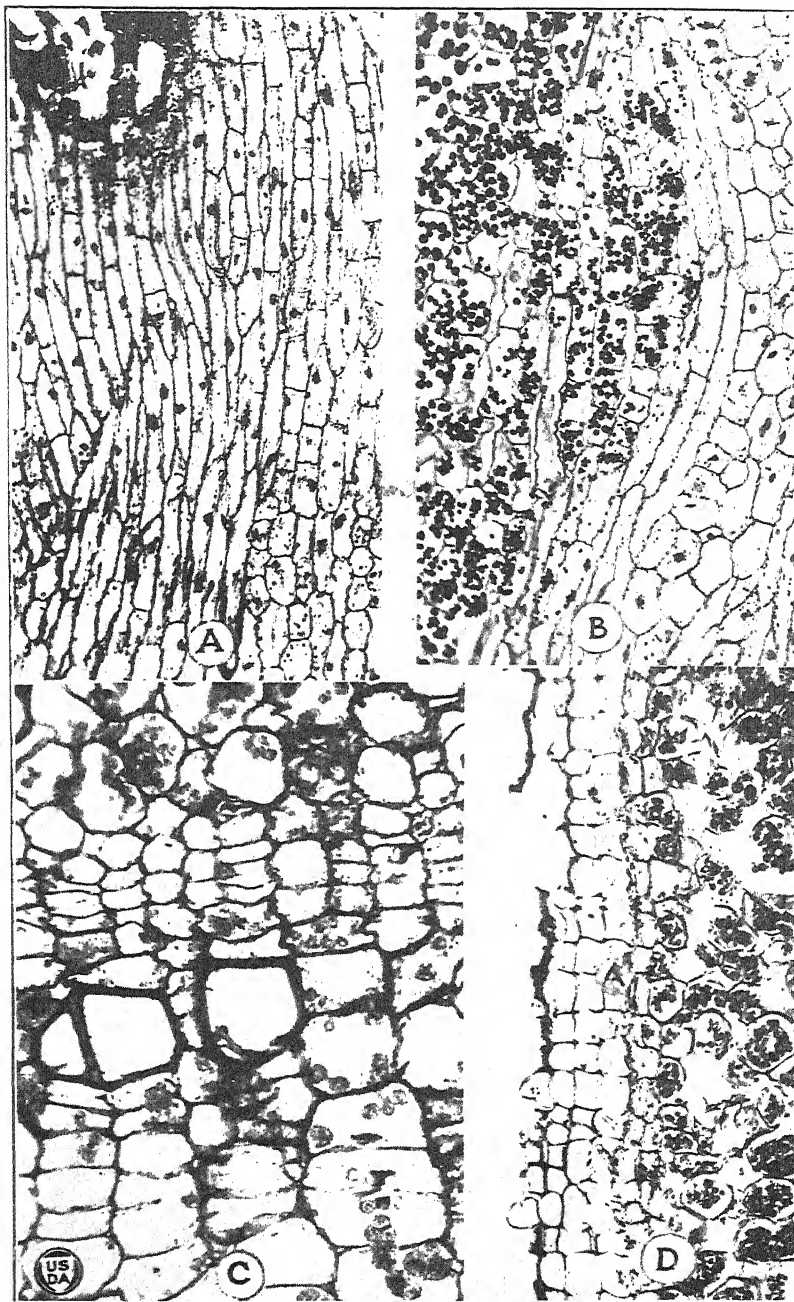


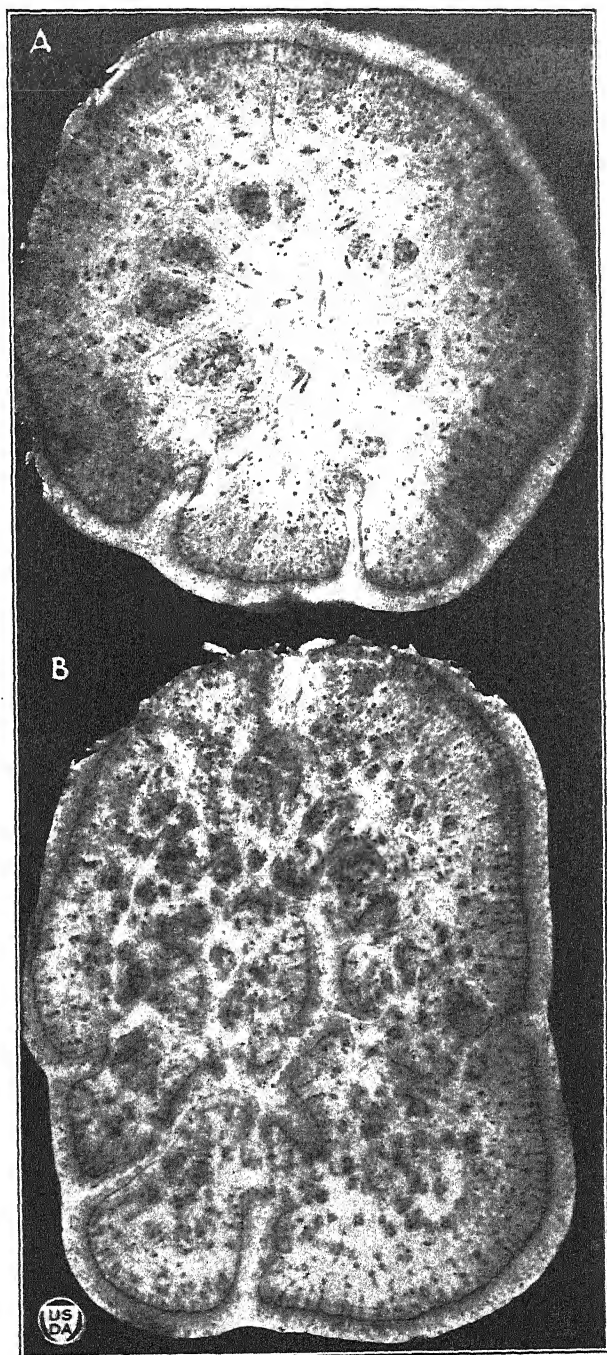
PLATE 2

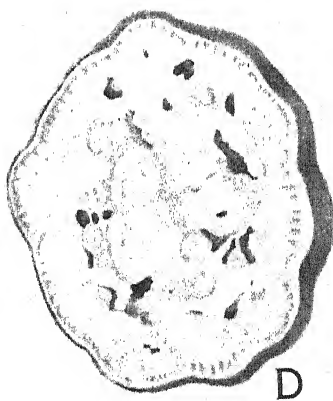
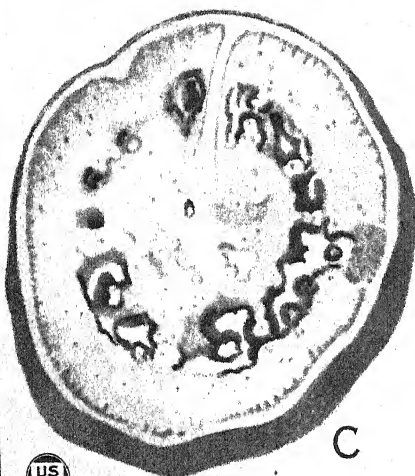
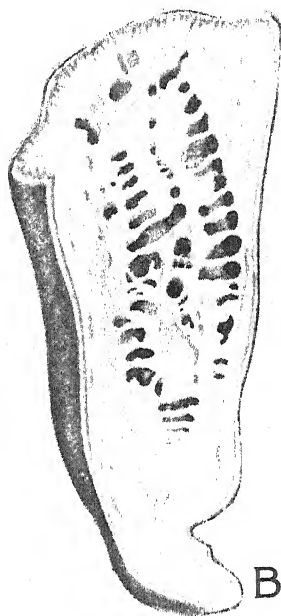
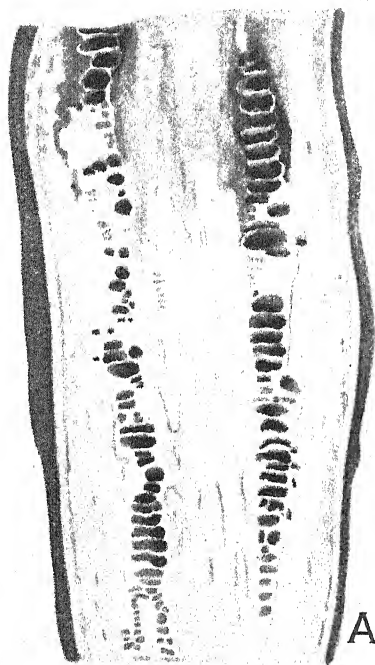
- A.—Tangential section of cambium of fleshy root.
- B.—Radial section through cortex and primary cambium. Notice the abundance of starch in the cells of the cortex.
- C.—Cross section through the primary cambium of a mature fleshy root. Notice the formation of a new, partial cambium below the group of xylem cells.
- D.—Longitudinal section of the periderm of a young fleshy root.

PLATE 3

A.—Cross section of a mature fleshy root (variety Belmont). Notice that the large center bundles are distinct.

B.—Cross section of a mature fleshy root (variety Nancy Hall). Notice that the larger bundles are united, forming a reticulum.







#### PLATE 4

A.—Radial cut of fleshy root (variety Key West) showing severe symptoms of internal breakdown. The longitudinal passages are interrupted by diaphragms. The lining of the cavities shows a brown discoloration.

B.—The same as above, except that the breakdown has not yet reached the stage showing brown discoloration. Cavities shaded in drawing.

C.—Cross section of fleshy root shown in A. Notice the denudation of the larger bundles.

D.—Cross section of fleshy root shown in B. This is a typical view exhibited by sweet potatoes affected with internal breakdown.



# THE INFLUENCE OF LOW TEMPERATURES AND OF DISINFECTANTS ON THE EGGS OF ASCARIS LUMBRICOIDES<sup>1</sup>

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## INFLUENCE OF LOW TEMPERATURES

The results of previous investigations have indicated that the eggs of *Ascaris lumbricoides* are resistant to low temperatures. Wharton (9)<sup>3</sup> states that low temperatures retard the development of the eggs but do not kill them; Yoshida (11) reports experiments in which the eggs were put on the ground or under a layer of soil and left through the winter months in Osaka, Japan, and their infectivity later proved by their being fed to guinea pigs with resulting infestation in these animals. Martin (7) states that the eggs resist freezing and are viable after being in the soil (Nebraska) over winter. In none of the above reports are definite temperature ranges given. Wigdor (10) kept eggs of *Toxascaris limbata* in the refrigerator at a temperature of 10° C. and found that their development was retarded but that it ultimately proceeded to the formation of motile embryos.

The present study was made to determine more definitely the possible effect which exposure to low temperatures may have on the normal development of the eggs of *Ascaris lumbricoides* from the pig. To determine the relative resistance of the eggs at different stages in their development, cultures of fresh, of partially developed, and of completely developed eggs (i. e., containing active embryos) were used. Such cultures were exposed to freezing temperatures above 0° F. (= -17.7° C.) and also below 0° F., and their subsequent development after restoration to 24° C. (75° F.) studied. The purpose of these tests was to determine the possibility of such eggs resisting such winter weather conditions as would prevail in pig pens.

## TESTS TO DETERMINE THE RESISTANCE OF FRESH ASCARIS LUMBRICOIDES EGGS TO VARYING FREEZING TEMPERATURES

Cultures of eggs were put in Petri dishes, just as squeezed from the uteri of the ascarids, were slightly moistened and put in freezers. The temperature was read two or three times each day.

The following exposures were made (1 day = 24 hours):

For 1 day below zero (-7° to -11° F.).....	culture 1.
For 2 days at zero (-2° to +2° F.).....	culture 2.
For 5 days:	
Above zero, temperature of 27-28° F.....	culture 3.
Above zero, temperature of 8-11° F.....	culture 4.
Below zero (-0.5° to -11° F.).....	culture 5.

<sup>1</sup> Accepted for publication Nov. 19, 1923.

<sup>2</sup> The experimental portion of this study was carried out in the chemical laboratory of Armour & Co. at Chicago, Ill., prior to the author's resignation from that company in October, 1919. This study was a joint project of Armour & Co. and the Federal Bureau of Animal Industry, Mr. Paul Rudnick and Dr. H. B. Raffensperger, respectively, directing the investigation. The writer takes this occasion to acknowledge her indebtedness to these gentlemen for their courteous assistance.

<sup>3</sup> Reference is made by number (italic), to "Literature cited", p. 175.

For 10 days:

Above zero (27.5° to 32° F.).....culture 6.

Below zero (0° to -16° F.).....culture 7.

For 20 days below zero (-14° to +5° F.)<sup>4</sup>.....culture 8.

For 30 days below zero (-14° to +5° F.)<sup>4</sup>.....culture 9.

For 40 days:

Above zero (10° to 18° F.).....culture 10.

Below zero (-2° to -16° F.).....culture 11.

After being thus exposed to freezing temperatures, the cultures were thawed, covered with potassium bichromate (10 per cent), and kept in an incubator at 24° C. In every case the eggs developed into active embryos, and neither the length of exposure to the cold nor the degree of cold itself seemed to affect the rate of development when the eggs were restored to 24° C. Cleavage appeared in 3 to 5 days, reached the many-celled stage in 6 to 8 days, and the motile embryos developed in 13 to 15 days. Although the low temperature in some cases seemed to break up the protoplasm of the egg so that it lost its normal appearance, this evidently did not interfere with the development, as cells with this appearance decreased in number or disappeared altogether during the incubation.

Although active embryos developed in all the tests, in the last, those for 40 days above and below zero F., the embryos seemed to be short-lived; on the sixth day after motion was first observed only a very few of the many embryos showed any activity. Apparently this prolonged exposure impaired the vitality of the worms, and it seems probable that the infectivity of such worms would be lessened.

#### TESTS TO DETERMINE THE RESISTANCE TO VARYING FREEZING TEMPERATURES OF PARTIALLY INCUBATED ASCARIS LUMBRICOIDES EGGS—THE EGGS SHOWING VARIOUS STAGES OF CELL DIVISION

*Ascaris lumbricoides* eggs, developed at 24° C. to various stages of cleavage, were held at freezing temperatures as follows:

For 7 days:

Above zero (8° to 12° F.).....culture A

Below zero (-2° to -16° F.).....culture A'

For 20 days:

Above zero (10° to 16° F.).....culture B

Below zero (-2° to -16° F.).....culture B'

After being kept at these freezing temperatures for the periods of time stated, the cultures were covered with a 2 per cent formalin solution and kept in the incubator (24° C.). All four cultures developed active embryos, and apparently all the eggs, at whatever stage of cleavage when put in the freezer, resumed their development when again incubated at 24° C. That the freezing shortened the life of these embryos was indicated, however, in the second set of tests, those for 20 days above and below zero F. The activity of the developed embryos was quite limited; 24 days after motion was first observed very few embryos could be found which showed any activity.

However, the viability of such embryos, developed from eggs which had been below zero F. for 20 days while in various stages of segmentation, was demonstrated in the following series (C) of tests—in culture F'; the embryonated eggs were fed to a guinea pig and an infection produced in the lungs.

<sup>4</sup> The temperature rose above zero for a brief period in the afternoon on three days.

# TESTS TO DETERMINE THE RESISTANCE TO VARYING FREEZING TEMPERATURES OF *ASCARIS LUMBRICOIDES* EGGS CONTAINING ACTIVE EMBRYOS

Two sets of cultures were kept in freezers, one above zero and one below zero, for the following lengths of time:

For 1 day:	
30° to 32° F.....	culture C.
-13° to -17° F.....	culture C'.
For 5 days:	
30° to 36° F.....	culture D.
-8° to -17° F.....	culture D'.
For 10 days:	
8° to 16° F.....	culture E.
-2° to -16° F.....	culture E'.
For 20 days:	
7° to 16° F.....	culture F.
-6° to -17° F.....	culture F'.
For 30 days:	
12° to 18° F.....	culture G.
-4° to -14° F.....	culture G'.

TABLE I.—Detailed tabulation of tests

State of egg before freezing.	Culture number.	Temperature to which exposed.	Period of time (days).	Result after restored to 24° C.
Fresh.....	I	-7° to -11° F.....	1	Developed into active embryos.
Do.....	2	-2° to +2° F.....	2	Do.
Do.....	3	27° to 28° F.....	5	Do.
Do.....	4	8° to 11° F.....	5	Do.
Do.....	5	-0.5° to -11° F.....	5	Do.
Do.....	6	27.5° to 32° F.....	10	Do.
Do.....	7	0° to -16° F.....	10	Do.
Do.....	8	-14° to +5° F.....	20	Do.
Do.....	9	-14° to +5° F.....	30	Do.
Do.....	10	10° to 18° F.....	40	Do.
Do.....	11	-2° to -16° F.....	40	Do.
Partially incubated...	A	8° to 12° F.....	7	Do.
Do.....	A'	-2° to -16° F.....	7	Do.
Do.....	B	10° to 16° F.....	20	Do.
Do.....	B'	-2° to -16° F.....	20	Do.
Containing active embryos.	C	30° to 32° F.....	1	Activity.
Do.....	C'	-13° to -17° F.....	1	Do.
Do.....	D	30° to 36° F.....	5	Do.
Do.....	D'	-8° to -17° F.....	5	Do.
Do.....	E	8° to 16° F.....	10	Do.
Do.....	E'	-2° to -16° F.....	10	Do.
Do.....	F	7° to 16° F.....	20	Do.
Do.....	F'	-6° to -17° F.....	20	Inactive.
Do.....	G	12° to 18° F.....	30	Active.
Do.....	G'	-4° to -14° F.....	30	Inactive.

All five cultures kept *above* zero F. for the stated periods showed active embryos after thawing. Of those kept *below* zero F. the first three (those of the 1-day, the 5-day, and the 10-day periods) showed active embryos. However, that the infectivity of these embryos was diminished was indicated by the fact that neither the culture kept below

zero F. for the 5-day nor the one for the 10-day period produced an infection of the lungs of a guinea pig when fed to it in large amounts. The embryos held for 20 days and for 30 days below zero F. showed no activity after thawing. The former culture (20-day), directly after thawing and when no activity could be seen, was fed to two guinea pigs; no migration of larvae occurred to the lungs of either. However, in this culture there were some eggs that were in the various stages of cleavage; these partially developed eggs formed active embryos when incubated after the freezing period, and these embryos proved infective when fed to a guinea pig. Nevertheless, the evidence showing diminishing vitality with lengthening exposure to low temperatures suggests that there might be a decreasing ability to develop beyond the larval stages to maturity in the normal host, but this is uncertain.

### THE INFLUENCE OF DISINFECTANTS

The following series of tests was made as a study of the manner in which the normal development of the eggs of *Ascaris lumbricoides* is affected by exposing them to chemical agents. Experiments by other investigators have shown that ascarid eggs are remarkably resistant. Several different ascarids have been used. Bataillon (1), experimenting with eggs of *Ascaris equorum* (= *Ascaris megalocéphala*), found that living embryos developed in eggs kept for six months in Fleming's solution and that these embryos remained motile and intact in 50 per cent alcohol 33 $\frac{1}{3}$  per cent acetic acid, and 20 per cent sulphuric acid. Of the dog and cat ascarids, the eggs of *Belascaris cati* (= *Ascaris mystax*) were found by Leuckart (6, p. 212) to develop completely in alcohol, chromic acid, and turpentine; Braun (2, p. 337) reports a similar resistance to those reagents and to a soda solution by the eggs of *Ascaris canis*, under which name he apparently includes several species. Wigdor (10) found that with the eggs of *Toxascaris limbata* nitric acid exhibited the highest ovicidal action of any of the acids, a 5 per cent solution destroying 50 per cent of the eggs, while the efficiency of sulphuric, hydrochloric, acetic, and oxalic acids decreased in the order given. The alkalis (slaked lime, caustic soda, ammonium hydroxid) were devoid of effect, as were also metallic and other salts (corrosive sublimate, copper sulphate, iron sulphate, sodium fluorid, and potassium arsenite), hydrogen peroxid, and a hypochlorite. The embryos were resistant to alcohols up to 70 per cent strength and to formaldehyde up to 35 per cent, but in more concentrated solutions and in volatile oils they either did not develop or were short-lived. The phenols, however, proved the most effective of any of the chemicals used by him, a 1 per cent solution of pure carbolic acid and similar dilute solutions of several commercial preparations entirely preventing the development of the ova.

The eggs of *Ascaris lumbricoides* exhibit a resistance similar to that of the above-mentioned species. With regard to the effect of acids, Galli-Valerio (3) found that embryos developed in solutions of sulphuric, hydrochloric, nitric, and acetic acids of 50 per cent or less in strength; more concentrated solutions proved more ovicidal, sulphuric acid being the most effective. Yoshida (11) found that embryos develop but soon die in eggs kept in 10-12.5 per cent solutions of sulphuric or acetic or 15-20 per cent hydrochloric acid; in a 1.5 per cent solution of nitric acid they were unharmed. Wharton (9) states that the embryos died in eggs kept in 0.5 per cent hydrochloric acid and in 3 per cent acetic acid. Of the

alkalis, ammonia was tested by Kobayashi (5). Yoshida (11) had found that eggs were unable to develop in urine, and according to Kobayashi this was interpreted by K. and S. Minagawa as the effect of ammonia formed from the urine. His results showed, however, that eggs survived more than a month in urine and for several days in ammonia (1 to 4 per cent). Salt solutions have little or no effect on the development of *Ascaris lumbricoides* eggs, Galli-Valerio (3) having obtained embryos in saturated solutions of copper sulphate, iron sulphate, copper acetate, and 50 per cent antiformin. He states that the eggs are destroyed after four months in pure antiformin. Ransom and Foster (8) found that the latter dissolved the shell of the egg but left a thin membrane around the embryo which would protect it from the action of the antiformin for at least five days. According to Kobayashi (4) eggs will develop in a saturated solution of sodium chlorid, but the embryos will die when they become mature.

Dilute formalin solutions and also a 10 per cent potassium bichromate solution have proved to be excellent culture media for *Ascaris* eggs, as they have no ovidical action and they keep the bacterial count low. According to Kobayashi (4) and also Yoshida (11), motile embryos will develop in a 10 per cent formalin solution but will not do so in solutions of 20 per cent or higher concentration.

Embryos in eggs kept by Ransom and Foster (8) in crude petroleum and in petrolatum were still active after 5 weeks; after 10 weeks those in the petrolatum were dead, but those in the crude petroleum still alive. With regard to the phenols, these writers state that they kept eggs of *Ascaris lumbricoides* containing embryos alive for several hours in carbolic acid. However, reports of Wharton (9) and Yoshida (11) were to the effect that the embryos do not develop in a 0.5 per cent solution. This is in accord with Wigdor's results, as stated above, with *Toxascaris* eggs.

The present study was made with phenol solutions in order to determine in great detail their ovidical properties and the practicability of their use as disinfectants in the control of *Ascaris*. It was felt that experiments, to be of practical value, must approximate conditions found in pig pens and that the disinfectant itself and the method of its application must be such as can be used there. Hence relatively pure cultures of eggs totally immersed in solutions of disinfectants would be valuable only to the extent of indicating the possibility of the use of these disinfectants under practical conditions. A disinfectant not proving efficacious under such ideal experimental conditions would certainly not prove so in practice. Therefore experiments were carried out with pure cultures covered with the disinfectant in a beaker and then those concentrations of the disinfectant which had proved ovidical under these conditions were used for further tests in which conditions were made to simulate those often found in pig pens of sanitary construction: the eggs were well mixed with sawdust and the latter sprayed with the disinfectant.

To determine the relative resistance of *Ascaris lumbricoides* eggs at different stages in their development, cultures of fresh, of partially developed, and of completely developed eggs (i. e., containing active embryos) were used. The cultures were prepared by squeezing the eggs out of the uteri of the worms, covering them with a 2 per cent formalin solution and keeping them in an incubator at a temperature of 20–24° C. until the desired stage of development was reached.

The two disinfectants used were carbolic acid and cresol, the latter in the official compound solution "liquor cresolis compositus." The formula<sup>5</sup> for this is—

Cresol .....	500 gm.
Linseed oil .....	300 gm.
Potassium hydroxid. ....	80 gm.
Alcohol .....	30 mils.
Water, sufficient quantity to make. ....	1,000 gm.

Unless otherwise stated, the exposure of the eggs to the disinfectant was carried out in a beaker, the eggs being covered with a deep layer of the solution and left for the stated time. They were then washed several times in water, put in Petri dishes with 2 per cent formalin and kept at 24° C. in the incubator. In the tests in which the eggs were mixed with sawdust the mixture was spread over the surface of a large flat pan and sprayed with an ordinary atomizer. After the treatment the eggs were recovered by washing the sawdust several times with water and centrifuging the washings; they were then kept in 2 per cent formalin at 24° C.

Two concentrations of each disinfectant were used: 5 per cent and 1 per cent of carbolic acid and 3 per cent and 1 per cent of cresol (6 per cent and 2 per cent of the compound). Both 5 per cent carbolic acid and 3 per cent cresol solution proved very effective in destroying the viability of the fresh eggs and of those in the various stages of cleavage and in destroying the activity of developed embryos. A 3 per cent cresol solution (6 per cent liquor cresolis compositus) accomplished this in 5 hours and a 5 per cent carbolic acid solution in 10 hours. In the tests in which the eggs were mixed with sawdust, thus obtaining conditions which may be found or readily established in pig pens of sanitary construction, it was found that when the mixture was sprayed once with either of the two disinfectants and then allowed to stand 24 hours, the ability of fresh eggs to develop and the activity of developed embryos were not always destroyed. However, if the sawdust mixture was thoroughly raked over and sprayed four times during the day and then allowed to stand over night, the treatment was more effective. Fresh eggs lost all vitality during such treatment with either disinfectant. With 3 per cent cresol all activity of developed embryos was destroyed, and although one active embryo was found directly after such treatment with 5 per cent carbolic acid, on the following day there was no activity. A repetition of such treatment on a second day assured the destruction of all vitality of fresh or developed eggs.

Fresh eggs and eggs at the intermediate stages of development were influenced to about the same degree by the disinfectants, although one test indicates that the further development of the latter is more easily destroyed than that of the former: 3 per cent cresol solution for 2 hours completely stopped the development of the partially incubated eggs, but did not do so with the fresh eggs.

Whereas in the freezing tests it was found that fresh eggs were more resistant to low temperatures than those containing active embryos, there are indications that the resistance of the two sorts of eggs may be reversed in regard to disinfectants: a 5 per cent solution of carbolic acid destroyed the vitality of fresh eggs in 5 hours, but embryos showed activity after such treatment, although their subsequent life was short.

<sup>5</sup> U. S. DISPENSATORY (20th edition), p. 631.



TABLE II.—*Tabulated summary of tests*

State of eggs.	Temperature.	Length of time in disinfectant.	Disinfectant used.			
			5 per cent carbolic acid.	1 per cent carbolic acid.	3 per cent cresol. (6 per cent liquor cresol. compositus).	1 per cent cresol. (2 per cent liquor cresol. compositus).
Fresh.....	24° C...	Hours. 2	A few embryos, but short lived.	Active embryos developed.	A few embryos developed.	Active embryos developed.
Do.....	...do....	5	No embryos developed.	...do.....	No embryos developed.	Do.
Do.....	...do....	10	...do.....	...do.....	...do.....	
Do.....	...do....	15½	...do.....	...do.....	...do.....	No embryos developed. Retest: Active embryos developed.
Do.....	...do....	20	A few embryos but short lived. Retest: No embryos developed.	...do.....	No embryos developed.	No embryos developed.
Do.....	...do....	24	(a) (Eggs under sawdust, sprayed once): A few embryos developed. (b) (Eggs mixed with sawdust, raked and sprayed 4 times): No embryos developed.	...do.....	(a) (Eggs under sawdust, sprayed once): No embryos developed. (b) (Eggs under sawdust, raked and sprayed 4 times): No embryos developed.	(a) No embryos developed. (b) (Eggs under sawdust, sprayed once): Active embryos developed.
Do.....	...do....	48	(a) No embryos developed; (b) (Eggs under sawdust, sprayed twice): No embryos developed; (c) (Eggs mixed with sawdust, sprayed 8 times): No embryos developed.	...do.....	(a) (Eggs under sawdust, sprayed twice): No embryos developed; (b) (Eggs mixed with sawdust, sprayed 8 times): No embryos developed.	(Eggs under sawdust sprayed twice): Active embryos developed.
Do.....	13° C...	5	Active embryos developed.	...do.....	No embryos developed.	
In intermediate stages of development.	24° C...	2			...do.....	
Do.....	...do....	5			...do.....	
Do.....	...do....	15½	No embryos developed.	Active embryos developed.		Active embryos developed.
Do.....	...do....	20	...do.....	...do.....		No embryos developed.
Do.....	...do....	24	...do.....			
Do.....	...do....	40		No embryos developed.		
Containing active embryos.	...do....	2	Activity.....		Activity.....	Activity.
Do.....	...do....	5	Slight activity; later none.		No activity.....	Do.
Do.....	...do....	8½	No activity.....			Do.
Do.....	...do....	15½	...do.....			Slight activity; later none.
Do.....	...do....	20	...do.....	Activity.....		No activity.
Do.....	...do....	24	(Eggs under sawdust, raked and sprayed 4 times): 1 active embryo found.		(Eggs under sawdust, raked and sprayed 4 times): No activity.	
Do.....	...do....	48	(Eggs under sawdust, raked and sprayed 8 times): No activity.		(Eggs under sawdust, raked and sprayed 8 times): No activity.	
Do.....	5-10° C.	5	Activity.....		Activity.....	
Do.....	...do....	16	...do.....		...do.....	

Cresol in the compound solution used proved to be more effective than an aqueous solution of carbolic acid; a 3 per cent cresol solution (6 per cent liquor cresolis compositus) had the same results on fresh eggs as a 5 per cent carbolic acid solution and was of greater value in the case of active embryos. A 1 per cent carbolic acid solution was too dilute to destroy the viability of fresh eggs when they were put in it for a 24-hour period; it is therefore not a satisfactory and practical disinfectant. A 1 per cent cresol solution (2 per cent liquor cresolis compositus) proved effective for fresh, partially developed, or fully developed eggs when used for a 20-hour period. In this instance, however, it is clearly shown how important is the distinction between the exposure as carried out with a pure culture in a beaker and, on the other hand, under conditions where the eggs are mixed with sawdust. In the latter case neither a thorough spraying with 1 per cent cresol and subsequent standing of the mixture for 24 hours, or even a repetition of the treatment on the second day destroyed the vitality of the fresh eggs. This weaker solution of cresol is therefore not so practical as the 3 per cent solution.

In regard to the relative efficiency of the disinfectants at 24° C. and at lower temperatures, the following was found to be true: 3 per cent cresol solution (6 per cent liquor cresolis compositus) worked as well at 13 to 15° C. as at 24° C. with fresh eggs (5 hours' exposure destroying their ability to develop), but with developed embryos, although at 24° C., it destroyed their activity in 5 hours, on the other hand, at from 5° to 10° C. it did not do so even in a 16-hour period. With carbolic acid a 5 per cent solution was not so effective on either fresh or developed eggs at refrigerator temperatures as at 24° C.

As to the value of disinfectants in destroying *Ascaris* eggs in pig pens, it would appear that proper cleaning of the pens is a more useful and more economical method of eliminating the eggs or reducing their numbers to a minimum than the use of disinfectants in the strengths and for the periods of time necessary to kill the eggs, assuming that thorough application of the disinfectants can be secured under practical conditions. The thoroughness of application necessary as indicated by the experiments is such that it would probably not be commonly secured in practice.

### CONCLUSIONS

Fresh and partially developed eggs of *Ascaris lumbricoides* show such very great resistance to low temperatures that subsequent development occurred even after the longest periods of exposure: With the fresh eggs a 40-day period at below zero Fahrenheit temperature (−2° to −16° F.) and with the partially developed eggs a 20-day period at the same temperatures. The life of the embryos that developed in these eggs was in both cases relatively short, but in the second case their infectivity for guinea pigs was demonstrated. Developed embryos in *Ascaris lumbricoides* eggs were killed by a 20-day exposure to such temperatures (−6° to −17° F.), but not by a 10-day period at the same temperatures nor by even a 30-day exposure to freezing temperatures above zero (12° to 18° F.). It is therefore evident that while very low temperatures may have a destructive effect upon the vitality of *Ascaris* eggs, many eggs under natural conditions are likely to survive severe winter weather, and the cold of winter can not be depended upon to destroy the vitality of *Ascaris* eggs present in pens, pastures, stables, etc. It does, however, diminish their infectivity in the course of time and may aid in controlling

infection by a mechanical action in holding eggs in frozen soil and thus inaccessible to swine.

Both an aqueous 5 per cent carbolic acid solution and 3 per cent cresol in a compound soap solution (*U. S. liquor cresolis compositus*) destroyed the capability of development in fresh and partially developed eggs and destroyed the activity of developed embryos. This was accomplished in 10 hours by the first disinfectant and in 5 hours by the second, when the eggs were completely immersed in the liquids. Under conditions as nearly analogous as possible to those in sanitary pens in which pigs are kept, the efficacy of the disinfectants was assured if the mixture of eggs and sawdust was thoroughly raked over and sprayed four times on each of two consecutive days. From these results it appears that phenol and cresol disinfectants may prove of value in helping to destroy *Ascaris* infection in pens and buildings under special conditions. The mechanical action of thorough scrubbing and cleaning and the heat of scalding water which should be used in cleaning are of great importance in the destruction of *Ascaris* eggs, and the destructive action of chemicals alone should not be expected to replace these measures.

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## THE BLACK-BUNDLE DISEASE OF CORN

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### INTRODUCTION

As the symptoms produced by organisms known to be parasites of corn became more clearly differentiated to the writers, there remained several groups of disease symptoms for which the explanations were meager or lacking. This paper deals with one of these groups of symptoms in a general way and specifically assigns an organism, not hitherto shown to be a parasite of corn, as one of the important causes.

### SYMPTOMS

Occasionally stripelike lesions are found in the leaves of young, infected corn plants (Pl. 6, A). Usually, however, black-bundle disease symptoms do not become evident during the first half of the growing season. During ear development various symptoms may appear as: Leaf and stalk color manifestations (Pl. 1), barren stalks (Pl. 2 A, B), nubbin ears (Pl. 2 B), prolific stalks (Pl. 2 C), or excessive suckering (Pl. 4 A, B). Such stalks usually show blackened fibrovascular bundles (Pl. 1).

In every field of dent, flint, and sweet corn (*Zea mays indentata*, *Z. mays indurata*, and *Z. mays saccharata*) under observation by the writers it has been noted that a number of plants have become red or purple on reaching the dough stage. The red coloration appears first at or near the midvein of the topmost leaf and progresses downward on the plant, affecting several leaves before progress on the stalk commences. In extreme cases the stalks and all the leaves become reddish purple, but all gradations between the initial appearance of the red color and this extreme condition may be the final color symptoms. Plants having any gradation of this reddening or purpling are designated in this paper as purple-leaf plants.

This type of reddening does not conform to any of those described by Emerson (14)<sup>2</sup> who says:

It is of interest to recall in this connection that plant colors of maize—brown no less than the red-purple series—develop first in the older parts where growth first ceases, such as the lower sheaths and the upper parts of the internodes of the culm.

However, a type of reddening, indistinguishable from the one encountered in commercial fields, sometimes occurs in selfed lines of dent corn. Inoculation of open fertilized dent corn with a particular organism, as will be shown later in this paper, increases the number of purple or red plants.

This disease is characterized also at this stage of development of the corn plant by high percentages of barren stalks and stalks producing

<sup>1</sup> Accepted for publication Nov. 24, 1923. The investigations here reported were conducted in cooperation with the Funk Bros. Seed Co., Bloomington, Ill., and the Wisconsin and Illinois Agricultural Experiment Stations.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 203.

nubbin ears only (Pl. 2, A, B). Often imperfectly developed ears are observed at a number of nodes on a stalk and more frequently multiple-ear production occurs at one node (Pl. 2, C), showing futile attempts at prolificacy. These manifestations, and large succulent stalks should be included, are the readily apparent symptoms associated with the black-bundle disease of corn and can be noted in any commercial field. When plants having any of these symptoms are cut open, blackened vascular bundles can nearly always be found in the nodes and internodes near the base and sometimes throughout the stalk (Pl. 1 and Pl. 3, B). Occasionally the fundamental tissue outside the blackened vascular tissue becomes browned or blackened, but usually in only one internode of a stalk.

However, the number of plants having these symptoms (excluding the black-bundle symptom) represents only a small part of the total number of plants affected by the disease. Plants, including the ears, may appear outwardly healthy and yet be infected from the crown to the tassel and every kernel on fine looking ears may have the organism internally. In these cases the presence of the black vascular bundles within the stalks is the most distinguishing symptom. The ears may be diagnosed by plating the kernels or by germination in conjunction with microscopic examination.

Apparently good seed ears selected from apparently normal stalks and producing 100 per cent of strong plants on the germinator may produce high percentages of purple plants in the field. No microscopic examination of seedlings on the germinator was made to determine infections. In these cases plant growth records during the first half of the season usually indicated strong rows. Table I shows in detail the record of an ear of the kind described and compares it with the records of four other ears. Among other things, it is an illustration of the statement that no appreciable indications of this group of symptoms are to be found during the first half of the growing season.

TABLE I.—Field performance of an infected ear row (row 3) compared with adjacent uninfected ear rows; Funk Ninety-Day corn planted May 24, 1919, in brown silt loam soil of medium fertility, at Bloomington, Ill.

Notations at different dates.	Condition of seed and accession numbers of seed ears.				
	Uninfected.		Infected.	Uninfected.	
	Row 1, No. 5794.	Row 2, No. 5831.	Row 3, No. 5824.	Row 4, No. 5887.	Row 5, No. 5897.
Stand and early vigor, June 13:					
Total stand.....	146	123	136	143	138
Per cent stand.....	97	82	91	95	92
Number strong plants.....	107	61	96	108	71
Number weak plants.....	6	11	12	7	5
Number blighted plants.....	1	3	0	1	1
General condition.....	Good.	Medium.	Good.	Good.	Good.
Condition, July 11 and 12:					
Color of foliage.....	Dark green.	Dark green.	Dark green.	Dark green.	Dark green.
Uniformity of growth.....	Uniform.	Variable.	Uniform.	Uniform.	Uniform.
Number leaning plants.....	3	3	2	22	16
Condition, Aug. 6:					
Number plants with wilting or dead leaves.....	3	15	5	3	27
Preharvest data, Sept. 11:					
Number good ears.....	131	99	40	132	117
Number mid-sized ears.....	1	2	10	1	6
Number nubbins.....	2	9	18	5	2
Number barren plants.....	6	22	54	2	9
Number smutted plants.....	6	7	7	3	2
Number prematurely dead plants.....	0	0	7	0	2
Percentage of purple plants.....	0	0	85	0	0
Acre yield in bushels.....	90	57.8	27.4	69.7	62

The seed ears referred to in Table I were the first generation crosses (artificially pollinated) of different strains of Funk Ninety Day, and when tested on the germinator, 30 kernels from each, showed 100 per cent vigorous seedlings and no evidence of rotting. Hence, it was thought that all were healthy. However, on September 11, row 3 (seed-ear 5824) was found to be uniformly diseased. As shown in Table I, there is a decrease in number of good ears and an increase in number of nubbins, barren stalks, and stalks with purple leaves, in comparison with adjacent rows on each side. Numerous stalks in row 3, when cut open at time of harvest, showed the characteristic blackened bundles. Usually in the latitude of central Illinois, the time of appearance of any easily recognized symptom of this disease is about the third week of August. At that time the first purple leaves usually appear, and barren and prolific stalks can be identified.

Symptoms as described and certain related observations have been noted for a number of years. For example, in the fall of 1917, an ear row of Funk Ninety-Day corn in a breeding plat showed certain outstanding characteristics. The plat was located on clover sod that had not grown corn for three years. The soil was uniform and of medium fertility. The ear from which the row in question was grown was of fine appearance in every respect and had shown strong germination on the germinator with no evidence of rotting. Hence it had been selected as a choice ear for breeding stock. During the early part of the season the plants showed no signs of disease. Attention was first directed to this row early in September following a light frost. All of the plants in this row were slightly injured by the frost, while the plants in adjacent rows showed no injury whatever. The row also contained an unusually large proportion of purpled plants and an abnormally large percentage of barren stalks. Further, on cutting open the stalks, it was found that most of the barren stalks and those with nubbins as well as some with good ears contained abundant blackened bundles. The row yielded only about one-half as much as the best adjoining rows. Seed ears for use in an experiment the following year (1918) were selected from this row, both from plants showing blackened bundles and from those which apparently were healthy. Eleven ears were thus selected, two from the apparently healthy plants and nine from the diseased ones. These ears were tested on the germinator in the usual way and all showed 100 per cent of strong germination, with no evidence of rotting. In 1918, these 11 ears were planted in ear rows along with 6 other ear rows from apparently healthy ears as controls. Unfortunately, the proportions of plants showing purple-stalk and black-bundle symptoms were not noted in 1918, but careful yield data were taken and computed to acre yields on a uniform moisture basis. The nine diseased rows produced an average of only 53.1 bushels per acre compared with 77.8 and 72.8 bushels per acre from the rows from the two specially selected ears and from the six control rows, respectively.

Certain earlier manifestations in the development of corn plants, such as abnormally fast growth in height or diameter, indicate that such plants later on are likely to produce symptoms of purple-stalk and black-bundle diseases. Some evidence that abnormally fast growth in height is an indication of disease was brought out from data obtained in 1919. Three hundred and twenty-seven plants were measured for height 36 days after planting and were grouped in quartiles according to height,

each quartile representing approximately one-fourth of the population. In order to determine the relation of height to production, the individual production of each plant was recorded in grams. The results are summarized in Table II and shown graphically in figure 1.

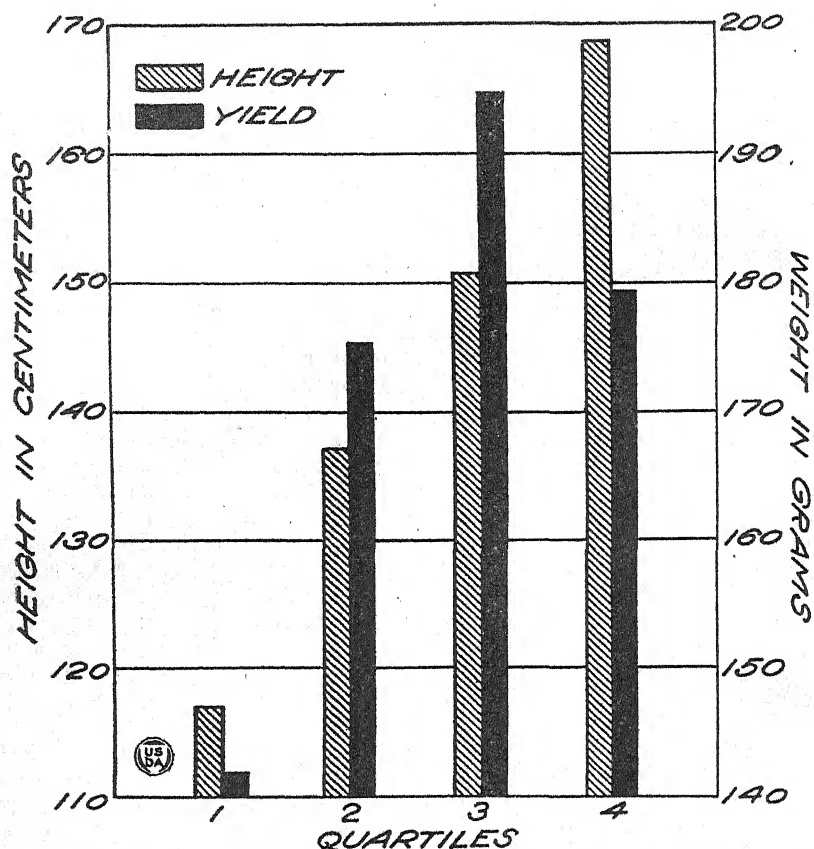


FIG. 1.—Graph showing average height of Yellow Dent corn plants, grouped in quartiles according to height, and average yield of plants in each quartile, respectively. Bloomington, Ill., 1919.

TABLE II.—Data showing average height of 327 plants of Yellow Dent corn, grouped in quartiles according to height, 36 days after planting, and average yield of plants in each quartile, respectively, grown on brown silt loam soil of high fertility near Bloomington, Ill., 1919 (see also fig. 1)

Quartile.	Number of plants.	Mean plant height.	Mean plant yield.	Increase in height (per cent).	Increase in production (per cent).
		<i>Cm.</i>	<i>Gm.</i>		
1.....	82	116.7	142.1		
2.....	82	137.1	175.6	Second quartile, 17.5...	Over first, 23.6.
3.....	82	150.6	194.4	Third quartile, 9.8.....	Over second, 10.7.
4.....	81	169.0	179.7	Fourth quartile, 12.2...	Over third, -7.6.



There is a direct relation between height of plant and production per plant except in quartile four which is made up of the plants of greatest height. This quartile contained a number of plants which grew abnormally fast. Also a higher percentage of barrenness was noted in this quartile. Briefly stated, in considering, during the first half of the growing season, a number of corn plants having the usual percentage of black-bundle disease, plant yield increases with plant height up to a certain point, and then decreases.

### HISTORY

While certain of the symptoms of this group, especially barrenness, has been extensively noted by various investigators, no comprehensive, detailed analysis or investigation of the causes of these abnormalities as a group has been made. Hunt (24, p. 151) says:

A varying percentage of the stalks of the field are barren—do not bear any ears. The percentage of barren stalks on a given soil varies with the thickness of planting and the season. Barrenness does not seem to be a variety characteristic. It seems to be largely the result of environment. If it were an hereditary characteristic the fact that the stalks are barren would tend to eliminate them.

Burt-Davy (6, p. 87-90) in considering barrenness as an hereditary character says that if barren stems were absolutely barren natural elimination would result, but the fact that they produce tassels and pollen lends color to the view that they may tend to reproduce their kind. He also mentions that cases are reported from America of 60 per cent of barren stems in a crop of maize. Blackwell (2, p. 20) in reporting the work of Hutchinson on South Carolina in using pollen from barren stalks to determine its effect on the amount of barrenness in the progeny, decided at first that it must be a Mendelian character and inherited in the ratio of 1 to 3; but later results did not bear out that conclusion. He adds that it has also been noted that there is or appears to be a correlation between barrenness and certain other characters, such as color, size of plant, shape of plant, length of life, and possibly others.

Pammel, King, and Seal, 1916 (37) described a cornstalk disease in Iowa due to *Gibberella saubinetii*. The stalks often were barren or had small ears. The small dwarf shoots in the axils of the leaves were frequently decomposed and the stalks were abortive. Hoffer and Holbert, 1918 (19) state that species of *Gibberella*, *Fusarium*, *Verticillium*, *Rhizopus*, and *Pseudomonas* are, in a great measure, responsible for missing hills, slow-growing stalks, barren stalks, down stalks, and early blighting of corn plants. They also say (20, p. 2, 11) that barren stalks and stalks bearing nubbins only seem to be correlated with certain pathologic conditions in the plants, \* \* \* that the rate of seedling development usually referred to as "vitality" is not a criterion for assuming freedom from infection of the seed by bacteria and species of *Fusarium*. The rate of seedling development on the germinator is not indicative of the yield possibilities of that seed ear. Selby, 1918 (46), says that rootrot shows in corn not only in weakening the roots, making it easy to blow over, but also in causing dwarfing of growth, premature dying of the top, and barrenness of stalks attacked. The diseased plants may be purplish-colored, dwarfed or stunted, and unproductive. Russell, 1919, (42) reports more barren stalks in dry years

such as 1913 and 1914. His observations during one of these years led him to believe the cause due to lack of plant food. The fact that the fields of corn observed were grown from seed from different sources was a factor possibly as important as lack of plant food. Hunt, 1919, (23) believes barren stalks are due to lack of fertility, a disease in the plant, or unfavorable weather conditions at time of pollination. Relative to diseases as the cause, he says:

Many stalks are pronounced barren when one or sometimes several shoots are found which started but failed to develop. If these shoots are examined they will be found to be infected with a mold which probably arrested the development of the ear.

Trost, 1922, (49) presented data which showed that 7.2 per cent of corn plants grown from horny seed and 11.4 per cent from starchy seed of the same strain of corn were barren.

Representative corn growers and breeders of Illinois have related the following observations:

Eugene D. Funk, of Shirley, Ill., writes in a letter of December 11, 1922:

The notes from our field men, as far back as 1901, show that we had more or less barren stalks in our breeding plats. At that time, however, we only made reference to the absence of ears and were of the opinion that the cause was due solely to heredity. More recent and careful study of the corn plant brought our attention to the color and development of the foliage, as well as the color of the stalk at harvest time, and now we definitely recognize that the purple, barren stalks are largely the result of a disease of the bundles.

Frank I. Mann, Gilman, Ill., writes in a letter of November 26, 1921:

If we had the purplish stalks and leaves in early times, I do not remember noticing them. For about 20 years, however, we have had many of them. As many of them produce very good ears, sometimes we could not associate this character with barrenness as fully as we do now, but still recognized it as very undesirable. There were a good many of these purple plants in our corn this fall. It seems to be the prevailing cause of complete and partial barrenness in our corn this season.

Harvey J. Sconce, Sidell, Ill., writes in a letter of December 5, 1921:

You are right in remembering that I had made a statement that in riding through cornfields on a horse or walking I had detected diseased corn plants several feet ahead of me. This was particularly the case again this year as the purple-stalk disease was quite heavy. They were in general all barren, had suckered badly, and every one was easily detected. I early learned to detect these plants as my breeding records date back to 1904 and as early as 1908 to 1910, I made notes on several rows that showed a greater percentage of these plants than other rows.

It is seen from the foregoing reports that both corn growers and investigators have described symptoms, such as barrenness, purple leaves and stalks, and production of nubbin ears. All of these symptoms fall into the group described herein and associated with the effects of parasitism by the organism discussed later. As these symptoms are often associated with other causes, the black-bundle symptom is chosen as the most distinctive, hence the common name.

In the early work on corn diseases by one of the authors (J. R. Holbert), the black bundles sometimes found in diseased corn were considered one of the symptoms of the corn root, stalk, and ear rots caused by species of *Fusarium*. This belief seemed to be shared by other workers. It was noted as early as 1917 that purple leaves and barrenness often were associated with the occurrence of black bundles. In the fall of 1919 the writers gave special attention to an ear row in which these symptoms developed in about 85 per cent of the plants. After examining the roots of some of the plants to a depth of 6 feet with negative results as to the presence of lesions of any importance, it was apparent that the cause

might not be due to species of *Fusarium*. Numerous isolations from the black bundles found in these plants consistently gave one organism, and pure-culture inoculations with it in the greenhouses at the University of Wisconsin in November, 1919, proved it to be pathogenic on corn. Without doubt this organism was responsible for the symptoms developed in the row in question. Subsequent investigations have shown, however, that symptoms almost identical with these may develop from other causes.

#### GEOGRAPHICAL DISTRIBUTION

The black-bundle disease of corn has been noted in Connecticut, New York, Ohio, Indiana, Illinois, Wisconsin, Minnesota, Iowa, South Dakota, Kansas, South Carolina, and California. This distribution renders it probable that it occurs wherever corn is grown in the United States.

#### ECONOMIC IMPORTANCE

The prevalence of this group of symptoms and their effect on productiveness of corn plants is such as to make it important that the causative agents be studied carefully and some effort made to lessen their occurrence. During 1919, 1920, and 1921, surveys of commercial fields near Bloomington, Ill., were made to determine the occurrence and productiveness of corn plants having the purple-leaf-and-stalk symptom. The results are summarized in Table III.

TABLE III.—*Occurrence of purple plants in some commercial fields of Yellow Dent corn near Bloomington, Ill., grown from seed selected from plants which showed no symptoms of the black-bundle disease*

Year.	Number of plants examined.	Purple plants (main stalks.)				
		Total.		Bearing.		Barren.
				Ears.	Nubbins.	
		Number.	Per cent.	Per cent.	Per cent.	Per cent.
1919.....	666	47	7.1	34.0	12.8	53.2
1920.....	4,857	351	7.2	27.9	31.9	40.2
1921.....	2,068	112	5.4	28.0	14.0	58.0

In addition to the preceding detailed observations, the authors have determined by count the percentages of purple-leaf plants occurring in a few scattered commercial corn fields in Indiana, Iowa, and Kansas, and at various places in Illinois. These have varied from a trace to 15 per cent. On the whole, however, it is believed that the data in the preceding table are fairly representative.

During 1920, in ear-row experimental plats of Yellow Dent corn at Bloomington, Ill., observations were made on 27 ear rows to determine the occurrence of purpling in main stalks and suckers. The relative number of purple-leaf plants which were barren or bore ears or nubbins also was noted. The seed ears were from plants that had not shown purpling in 1919. In planting the plats, twenty kernels were planted singly 14 inches apart in rows 42 inches apart. The results are given in Table IV.

TABLE IV.—Data on the occurrence of purple-leaf symptoms developed in experimental plots of Yellow Dent corn grown in 1920 near Bloomington, Ill., from seed ears selected from plants not showing purple-leaf symptoms, the seed being untreated

Row No.	Hills, total number.	Suckers.		Plants (main stalks) without purple leaves.	Plants (main stalks) with purple leaves.			
		Total.	Purpled.		Total number.	Bearing.		Barren.
						Ears.	Nubbins.	
1.....	15	25	2	12	3	2	1	0
2.....	14	25	3	12	2	2	0	0
3.....	16	26	2	15	1	1	0	0
4.....	11	13	1	10	1	1	0	0
5.....	15	21	0	15	0	0	0	0
6.....	17	33	4	15	2	1	0	1
7.....	17	21	0	16	1	0	1	0
8.....	15	18	7	14	1	1	0	0
9.....	16	19	0	16	0	0	0	0
10.....	13	30	1	12	1	1	0	0
11.....	19	29	1	18	1	0	1	0
12.....	17	26	4	16	1	1	0	0
13.....	5	8	0	5	0	0	0	0
14.....	17	16	2	17	0	0	0	0
15.....	17	11	0	17	0	0	0	0
16.....	17	39	0	17	0	0	0	0
17.....	19	44	9	17	2	1	1	0
18.....	15	29	3	15	0	0	0	0
19.....	16	28	2	16	0	0	0	0
20.....	17	38	6	15	2	0	2	0
21.....	18	24	3	18	0	0	0	0
22.....	18	35	6	16	2	1	1	0
23.....	13	28	1	13	0	0	0	0
24.....	12	27	0	12	0	0	0	0
25.....	11	28	6	10	1	1	0	0
26.....	16	35	1	15	1	1	0	0
27.....	12	25	2	10	2	1	1	0
Total..	408	701	66	384	24	15	8	1

Table IV presents data which, when expressed in percentages, show that 5.9 per cent of the plants had the color symptom that was used as a basis in these data, and the relative percentages of these plants that were barren (4.2), nubbin-producing (33.3), and ear-producing (62.5).

In 1919, ears of Yellow Dent corn were selected from purple plants with a view to determine their field performance in 1920. In planting the plots, 40 kernels were planted singly 14 inches apart in rows 42 inches apart. The results from 23 of these ears are given in Table V.

On the basis of the data presented in Table V, it is found: (1) That 46.6 per cent of main stalks and 43.2 per cent of all suckers in these rows showed the purple-leaf symptoms; (2) that of the plants with the purple-leaf symptom, 55.1 per cent produced ears, 27.5 per cent produced nubbins, and 17.4 per cent were barren; and (3) that the mean yield of plants showing the purple-leaf symptom was 176 gm. per plant as compared with 264.2 gm. per plant from apparently healthy plants in the same ear rows, thus showing 33.4 per cent reduction in yield per plant by those plants having the purple-leaf symptom.

Along with the experiments referred to in Tables IV and V, another experiment was conducted in 1920 to determine if seed treatment with

corrosive sublimate would eliminate the purple-leaf symptoms. In the matter of control the results were almost negative, but the data obtained were important in other ways, and hence they are included at this point.

The ear rows were planted with seed from the same ears as those used for the experiment referred to in Table V, which were selected from purple-leaf plants. The kernels were planted singly 14 inches apart in rows 42 inches apart. This seed was treated by immersion for one-half hour in a 1 : 1000 mercury bichlorid solution. The results are given in Table VI.

TABLE V.—Data on the occurrence of purple-leaf symptoms and on yield of Yellow Dent corn grown near Bloomington, Ill., 1920, in ear-row experimental plats, from seed ears selected from plants showing purple-leaf symptoms in 1919, the seed used in 1920 being untreated

Row No.	Number of—			Plants without purple-leaf symptoms.				Plants with purple-leaf symptoms.						
				Number of—		Yield.		Number of—		Yield.		Number producing—		
	Plants.	Suckers.	Purple suckers.	Plants.	Ears.	Total.	Average per plant.	Plants.	Ears.	Total.	Average per plant.	Ears.	Nubbins.	Barren.
						Gm.	Gm.			Gm.	Gm.			
1.....	22	44	25	11	11	2,761	251	11	10	2,080	189	7	3	1
2.....	25	31	24	11	11	2,448	223	14	9	2,012	144	5	4	1
3.....	27	31	10	19	20	4,848	255	8	7	1,402	175	4	3	1
4.....	31	47	28	7	7	1,944	278	24	19	3,418	142	10	9	5
5.....	25	38	19	8	8	2,068	259	17	15	2,840	167	8	7	1
6.....	32	43	11	20	20	5,661	283	12	10	2,268	189	7	3	2
7.....	20	36	18	8	8	2,071	259	18	16	3,288	183	10	6	2
8.....	25	26	15	12	12	3,172	264	15	9	1,942	149	6	3	4
9.....	31	51	15	19	22	5,600	295	12	14	3,055	255	9	1	1
10.....	29	22	12	17	17	4,766	280	12	12	2,192	183	7	5	0
11.....	29	30	18	14	14	3,972	284	10	9	2,273	227	8	1	0
12.....	29	43	25	7	7	2,000	286	22	20	4,044	184	16	4	3
13.....	32	43	18	18	18	4,519	251	14	14	2,636	188	7	7	0
14.....	28	30	11	18	18	5,253	292	10	6	1,386	139	4	2	4
15.....	17	10	2	74	14	4,098	293	3	3	792	264	2	1	0
16.....	30	40	17	19	19	5,548	292	11	9	2,509	228	9	0	2
17.....	31	44	7	25	26	7,255	290	6	4	993	166	4	0	2
18.....	34	26	8	18	18	4,172	232	16	13	3,011	188	7	6	3
19.....	11	12	3	9	9	1,873	208	2	2	200	100	1	1	0
20.....	16	9	3	9	9	2,228	248	7	6	1,134	162	4	2	1
21.....	15	18	11	3	3	672	224	12	7	1,600	133	5	2	5
22.....	15	15	0	12	12	3,494	291	3	2	214	71	1	1	1
23.....	14	15	6	6	6	1,426	238	8	6	1,340	168	5	1	2
Total.....	569	704	304	304	309	81,849	264.2	265	222	46,629	176	146	73	46

Table VI shows that, although treated for one-half hour in a 1:1000 solution of mercury bichlorid, a high percentage (42.7 per cent) of purple plants resulted from planting seed selected from purple plants and that purple plants yielded less (20.6 per cent) than apparently normal plants from the same ears. A comparison of this table with Table V is shown in Table VII.

It will be noted from the summaries given in Table VII that, although no important effect resulted from this treatment, there was a slight decrease in the percentage of plants with the purple-leaf symptom and an increase in the yields of both healthy and diseased plants. In general, however, the results show the desirability of selecting seed ears only from plants not showing the purple-leaf symptom. This statement is

made because the data show that ears selected from such diseased plants produced 46.6 and 42.7 per cent plants with the purple-leaf symptom, respectively, as compared with 5.9 per cent plants with the same symptom in corresponding ear rows from seed selected from apparently healthy plants (Table IV).

TABLE VI.—Data on the occurrence of the purple-leaf symptom and on yield of Yellow Dent corn grown near Bloomington, Ill., 1920, in ear-row experimental plats from seed ears selected from plants having the purple-leaf symptom in 1919, the seed being treated with 1:1000 mercury bichlorid solution for one-half hour immediately before planting (1920)

Row No.	Number of—			Plants without purple-leaf symptoms.				Plants with purple-leaf symptoms.								
	Plants.	Suckers.	Purple suckers.	Number of—		Yield.		Number of—		Yield.		Number producing—				
				Plants.	Ears.	Total.	Average per plant.	Plants.	Ears.	Total.	Average per plant.	Ears.	Nubbins.	Barren.		
						Gm.	Gm.			Gm.	Gm.					
1.	11	17	9	5	5	1,470	294	6	5	1,762	127	2	3			1
2.	14	15	11	7	7	1,856	265	7	5	1,264	181	3	2			2
3.	17	30	6	11	11	2,614	238	6	5	1,144	191	4	1			2
4.	17	19	12	7	7	1,754	251	10	7	1,454	145	5	5			3
5.	16	26	13	3	3	800	267	13	12	2,202	169	6	6			1
6.	16	24	8	10	16	2,713	271	6	5	1,166	194	3	2			2
7.	16	12	2	12	12	3,086	257	4	4	1,188	297	3	1			1
8.	15	23	16	7	7	2,165	309	8	7	2,296	287	7	0			1
9.	16	20	13	7	7	2,090	299	9	9	2,372	264	8	1			1
10.	15	9	0	12	12	3,372	281	3	3	746	240	3	0			1
11.	11	9	4	9	9	2,788	310	2	1	304	152	1	0			1
12.	15	17	13	11	11	3,026	275	4	3	810	203	3	0			1
13.	16	13	5	7	7	1,484	212	9	8	1,786	198	4	4			1
14.	11	14	3	10	10	3,488	349	1	1	290	290	1	0			1
15.	6	2	0	5	5	1,612	322	1	1	346	346	1	0			1
16.	18	27	6	14	14	4,320	309	4	4	1,122	281	3	1			1
17.	16	12	4	12	12	3,170	264	4	4	1,192	298	4	0			1
18.	18	14	3	11	11	2,950	268	7	5	1,198	171	3	2			2
19.	14	18	15	4	4	940	235	10	9	1,688	169	4	5			1
20.	13	8	5	6	6	1,450	242	7	7	1,104	158	2	5			1
21.	17	27	12	8	8	2,098	262	9	7	1,730	192	6	1			1
22.	15	16	4	11	11	2,980	271	4	4	1,072	268	4	0			1
23.	14	24	15	4	4	934	234	10	8	2,032	203	6	2			2
Total.....	337	396	179	193	199	53,160	275.4	144	124	29,268	218.8	86	38			20

TABLE VII.—Summary of data given in Table V, where seed was untreated, and Table VI, where seed was treated with mercury bichlorid solution 1:1000 for one-half hour. The seed in both cases was from the same ears selected from purple-leaf plants

Points of comparison.	Table V, seed untreated	Table VI, seed treated.
Percentage of all plants that showed purple-leaf symptoms.....	46.6	42.7
Percentage of all suckers that showed purple-leaf symptoms.....	43.2	45.2
Average yield of apparently healthy plants.....	264.2	275.4
Average yield of purple-leaf plants.....	176.0	218.8
Average percentage reduction in yield from purple-leaf plants..	33.4	20.6
Percentage of plants with the purple-leaf symptom that produced ears.....	55.1	59.7
Percentage of plants with the purple-leaf symptom that produced nubbins.....	27.5	26.4
Percentage of plants with the purple-leaf symptom that were barren.....	17.4	13.9

During the same year (1920) an experiment was conducted to compare plant yields of corn grown from ears selected in 1919 from purple-leaf plants and corresponding ones from plants not showing this symptom. Twenty-seven ear rows of equal length (25 feet) were planted from each lot of ears. Kernels were planted singly, 14 inches apart in rows 42 inches apart. The results are given in Table VIII.

TABLE VIII.—Comparative yields of 25-foot ear rows of Yellow Dent corn, grown near Bloomington, Ill., in 1920, from ears selected in 1919 from purple-leaf plants and from plants without the purple-leaf symptom

Row No.	Seeds selected from purple-leaf plants.		Row No.	Seeds selected from plants without purple-leaf.	
	Number of plants.	Yield.		Number of plants.	Yield.
		Gm.			Gm.
1.....	11	2,136	9B.....	15	4,899
2.....	11	2,300	9C.....	17	3,460
3.....	11	2,320	9T.....	15	4,360
4.....	16	2,132	18B.....	16	3,682
5.....	16	2,602	18C.....	17	3,650
6.....	15	3,206	18T.....	15	4,534
7.....	12	2,590	27C.....	18	3,973
8.....	14	2,968	27T.....	18	3,917
9.....	14	3,422	28.....	15	3,777
10.....	15	4,658	29.....	14	4,554
11.....	12	3,606	30.....	16	4,162
12.....	12	2,724	31.....	11	3,660
13.....	18	4,146	32.....	15	3,689
14.....	14	3,744	33.....	17	4,144
15.....	6	1,880	34.....	17	3,946
16.....	16	4,424	35.....	15	3,962
17.....	14	4,218	36.....	16	4,086
18.....	17	3,524	36C.....	16	5,020
19.....	11	2,073	36T.....	15	3,775
20.....	16	3,362	39.....	16	4,313
21.....	15	2,272	39T.....	14	3,264
22.....	15	3,708	41.....	15	3,718
23.....	14	2,766	41T.....	15	3,859
24.....	15	4,084	45C.....	18	3,945
25.....	19	5,016	45T.....	15	3,434
26.....	16	3,490	50T.....	15	3,717
27.....	15	2,413	54C.....	20	3,495
Average yield per row.....3,181±123.0			Average yield per row.....3,959±54.3		

On the basis of the data given in Table VIII it will be noted that the average yield from the 27 rows from seed of purple plants was  $778 \pm 134.5$  gm, less than the average yield from the corresponding ear rows grown from seed selected from plants without the purple-leaf symptom. This is a 19.7 per cent reduction in yield, and is significant in terms of the probable error involved.

#### CAUSES

All purple, barren, nubbin, prolific, or multiple-eared plants, or those with blackened bundles fall into the group under discussion. It should be mentioned that these symptoms rarely occur singly, but are grouped



in various ways in single plants. Indications are that certain of these symptoms may result from different causative agents and also that all of them may be caused by one agent.

Lack of pollination results in barren plants no matter what the cause, and usually all barren plants observed in commercial fields turn purple. Corn plants attacked by smut often turn purple and are sometimes barren. The physiologic factors accompanying loss of vigor by selfing sometimes bring out this color symptom. Other factors disturbing the natural physiologic processes of corn plants at critical stages in their growth may cause purple colorations. For example, one of these may be demonstrated by breaking off the ears when in the milk stage. An experiment bearing on this was conducted in 1921, the results of which are given in Table IX.

TABLE IX.—Number and percentage of Yellow Dent corn plants that turned purple following removal of ears when in milk stage in comparison with uninjured plants in corresponding row, the corn being planted May 27, 1921, near Bloomington, Ill., de-eared August 10, and data recorded September 9

Rows.	Plants.		
	Total number.	Purpled.	
		Number.	Per cent.
Not de-eared.....	272	11	4.0
De-eared.....	272	118	43.4

In the progress of the investigations it has been found that the fungus *Cephalosporium acremonium* Corda (9, p. 11) is one of the most important causes in producing the group of symptoms discussed earlier. Occasionally bacteria are found associated with *C. acremonium* in the infected bundles, especially in the lower internodes. In fact, at first it was suspected that bacteria might play an important part in causing certain of these disease manifestations. Even yet, the exact status is not fully clear in this respect, but is being investigated further.

Whether or not the bacteria play a part in the complex, it has been found that *Cephalosporium acremonium* by itself is definitely a parasite capable of producing characteristic symptoms, and that it is seed-borne.

In 1919 special attention was turned to this group of symptoms and *Cephalosporium acremonium* was isolated consistently from blackened bundles of purple-leaf plants. Inoculations with this organism at the bases of dent, flint, and sweet corn when about a foot in height caused blackened bundles in the green leaves and in the stalks. From these leaves and stalks isolations were made and identified as the organism used in the inoculations. Inoculations in the field at Bloomington, Ill., and in the greenhouses at the University of Wisconsin and the University of Illinois in the following winter proved beyond doubt the pathogenicity of this organism; but not until 1921 were any inoculations made with pure cultures in the field from which reliable yield data could be secured. The methods of inoculation and the results are presented in another part of the paper.



In the light of subsequent experience the consistent association of this organism with purple-leaf plants in 1919 was due no doubt to the fact that all the material was collected at one place, Bloomington, Ill., and that practically all of the purple plants produced there in that season were due to the effect of this organism. No such consistency of association was found during 1920 from material collected at the same place, but again in 1921 *Cephalosporium acremonium* was found to be present in a high percentage of isolations from plants of both dent and sweet corn having black vascular bundles. The group of symptoms under discussion may occur to some extent without the presence of *C. acremonium*. Only careful examinations and analyses can determine when this organism is responsible for these symptoms. It is also true that infections by this organism may be quite general in corn without the presence of any of these symptoms to a marked extent, except the blackened fibrovascular bundles.

#### THE ORGANISM

The genus *Cephalosporium* Corda (9, p. 11) is characterized by well-developed hyaline mycelium and slender unbranched conidiophores, the spores of which are borne singly at the apex but are pressed to the side by the next produced and eventually form a head held together by mucilage. According to Buchanan (5) the genus *Hyalopus* Corda is differentiated from *Cephalosporium* solely by the more abundant production of mucilage by the former and the resultant globular refractive head produced. He also concurs with Lindau's (28, p. 100-101) characterization of *Hyalopus* as a *Cephalosporium* grown in a moist atmosphere, and he also thinks it possible that *Allantospora* Wakk. is but a growth form of *Cephalosporium*. Massee (33, p. 274, 292), (fig. 2), quoting from Grove (18), distinguishes *Cephalosporium* from *Botryosporium* by the creeping primary hyphae and by the absence of distinct conidiophores at the tips of the branchlets. It differs from *Acremonium* by having more than one spore borne at the end of each conidiophore.

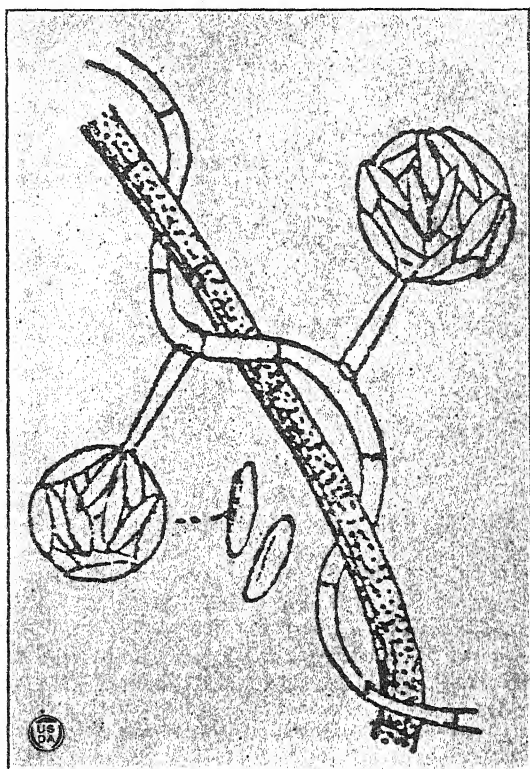


FIG. 2.—After Massee (33, p. 274, fig. 23) *Cephalosporium acremonium* twining around a black mold.

*Cephalosporium acremonium* Corda (9), (fig. 3), was described and figured by Corda in 1839, giving it somewhat wide limitations so that it includes any *Cephalosporium* with small simple oblong or ovoid spores. He found it on dead insects and fungi. The spores figured by him appear too broad to be identical with the species described later and indicate that the organism dealt with in the original description may be identical with *C. acremonium* form *major* described by Penzig (38) and later by Grove (18). The spore measurements reported for the species

and subspecies of *C. acremonium* are presented in Table X.

Dietrich, 1848 (12, p. 157), and Bonorden, 1851 (3, p. 108), gave almost the same description and like Corda did not give spore measurements. The first report of the fungus on corn was by Fresenius in 1863 (17, p. 94-95), (fig. 4), who reported it on the drying leaves. He gave a careful description, spore measurements, and figures, and retained the name *C. acremonium*. Since his description is entirely in accord with the findings of the authors, a translation of it is presented later in this paper. Rivolta (41) in 1873 figured it together with other fungi in his study of plant parasites in their influence on digestion in domestic animals. Saccardo, 1878 (44, p. 271), reported it parasitic on *Hypoxylon purpurea*. Penzig, 1882 (38), re-

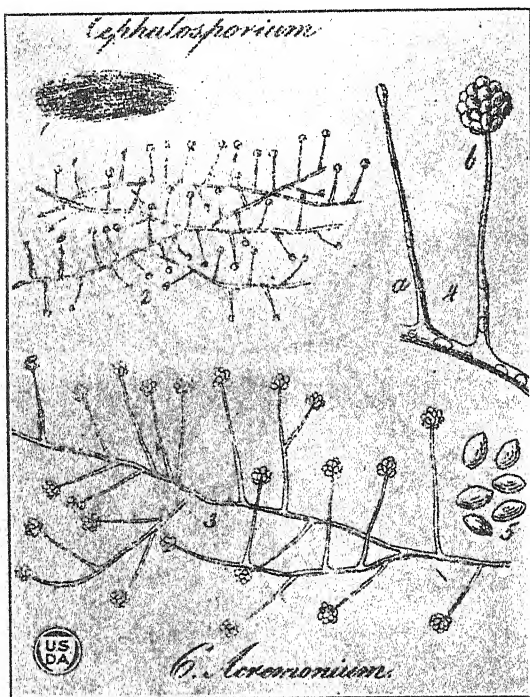


FIG. 3.—After Corda (9, t. 3, p. 11, pl. 2, fig. 29)

1. Colony.
2. Few plants much enlarged.
3. Single plant greatly enlarged.
4. Piece of hypha with two sporophores, one bearing a spore-head greatly enlarged.
5. Spores greatly enlarged.

ported a *Cephalosporium* parasitic on *Alternaria tenuis* in leaves of *Citrus aurantium* and classified it as *C. acremonium* Corda form *major* Penzig. He thought it probably identical with *Acrostalagmus albus* Pr. The latter (40, p. 126) was found on dead insects in leaves of *Citrus limonum*. Saccardo, 1881 (43) and 1886 (45), Costantin, 1888 (10, p. 91), Lindau, 1889 (26, 27, 28), Sorauer, 1908 (48), Ferraris, 1910 (16, p. 615), and Oudemans, 1919 (35, p. 692), described and figured or gave references to this fungus. Lindau named another fungus host, *Chloridium giganteum*. Massee, 1887 (32), found a *Cephalosporium* parasitic in hyphae of *Heterosporium colocasiae* and named it *C. acremonium* Corda var. *uniseptatum* Massee. Oudemans and Koning, 1902 (36), found *C. acremonium* in forest humous soil in Holland. Their careful descrip-

tion is published in English by Jensen, 1912 (25). Dop, 1906 (13), described a *Cephalosporium*like fungus upon the insect *Aspidiotus perniciosus* on the leaves of *Cocos nucifera* in the island of Martinique. He named it *Hyalopus yvonis*. The description and spore measurements are in accord with *C. acremonium*. Bainier, 1907 (1), cultivated, described, and figured the fungus, as did Dale, 1914 (11, v. 12, p. 56), in working with soil fungi. She thinks also that the form determined in her first paper (11, v. 10, p. 465) as *Verticillium* is really *C. acremonium*. Peyronel, 1914 (39), found *C. acremonium* now and then in the air on high mountains. Fawcett, 1915 (15), reported a *Cephalosporium* sp. as the cause of "zoned" leaf spot of coffee. Zimmerman, 1916 (50), reported the fungus as a parasite of scale insects on coffee but later gave it the name of *C. lecanii*. Ciferri in Italy, 1921 (8), reported *Aspergillus varians* growing on kernels of maize that had developed on the extremities of the ears of plants growing in soil of low humidity. *C. acremonium* was frequently found by him as a parasite on the *Aspergillus*. Manns and Adams, 1921 (29, 30, 31), made investigations upon fungi carried within the kernels of seed corn. They found that 39.54 per cent of Delaware seed corn for planting in 1921 was affected internally with a species of *Cephalosporium* which they determined as *C. sacchari* Butler and Khan. Butler and Khan (7) described *C. sacchari* as the cause of a wilt of sugar cane in India. The spores of this species are larger than those of *C. acremonium* and often become 1 to 3 septate before germinating. The authors found, upon examination of cultures kindly supplied by Manns and Adams, that the Manns and Adams *Cephalosporium* does not correspond to the species *sacchari* but to *acremonium* as described by Fresenius (17, p. 94-95), and therefore to the organism with which the authors are working. Branstetter, 1922 (4), followed Manns and Adams in the identification of the fungus on corn as *Cephalosporium sacchari*. Relative to the fungi carried within the kernels, he says:

The disease survey shows that 1921 Missouri corn was heavily infected with *Fusarium moniliforme*, *Cephalosporium sacchari* and *Diplodia zeae* in the order named.

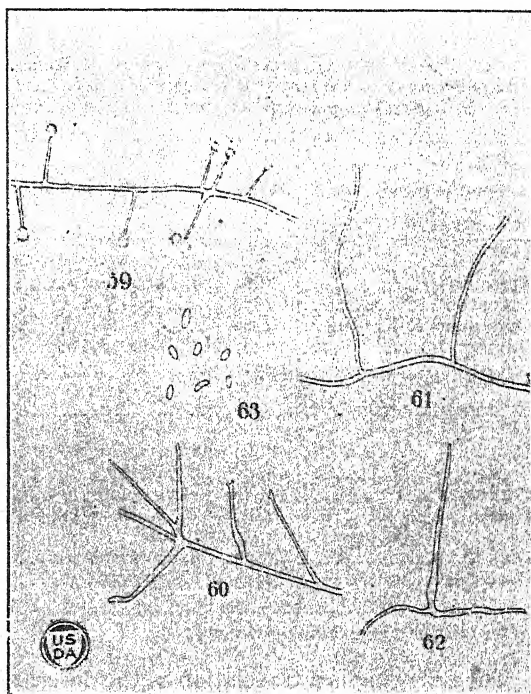


FIG. 4.—After Fresenius (17, Hft. 3, pl. 11, fig. 59-62).

59. Piece of mycelium with sporeheads.  
60-62. Mycelium with conidophores but no spores.  
63. Spores.

He also states that both *F. moniforme* and *C. sacchari* grew out of many platings and that in these cases only the organism that appeared first was considered. As *F. moniliforme* is a faster-growing organism than the so-called *C. sacchari*, the above statement on the relative occurrence of these two organisms may be misleading.

In comparing closely related species the size of the spores is a very important character. For this reason the spore measurements of *C. acremonium*, *C. sacchari*, *Acrostalagmus albus*, and *Hyalopus yvonis* are presented in Table X, which also includes the names of the investigators making the measurements and the dates of publication.

TABLE X.—Names of investigators, dates of publications, and spore measurements recorded by each author for a number of species and subspecies of *C. acremonium*, *C. sacchari*, *Acrostalagmus albus*, and *Hyalopus yvonis*

Name of investigator.	Date.	Name of organism.	Spore measurements.	
			Length.	Breadth.
Fresenius .....	1863	<i>C. acremonium</i> .....	Microns. 3.3-6	Microns. .....
Saccardo .....	1878	.....do.....	4	1
Oudemans & Koning ..	1902	.....do.....	4	1-1.5
Bainier .....	1907	.....do.....	2.5-5	.....
Ciferri .....	1921	.....do.....	4.8-6	1.8
Estimated average.....			4.5	1.35
Penzig .....	1882	<i>C. acremonium form major</i> .....	4.5-5	2-2.5
Grove <sup>a</sup> .....	1893	.....do.....	4-5	2
Preuss .....	1882	<i>Acrostalagmus albus</i> .....	3.3-3.5	1-1.5
Massee, G. ....	1887	<i>C. acremonium form uniseptatum</i> .....	10	4
Dop, P. ....	1906	<i>Hyalopus yvonis</i> .....	4	1-1.5
Butler & Khan .....	1913	<i>C. sacchari</i> .....	4-12	2-3

<sup>a</sup> Spores more oblong than Corda's figures (6).

The following is a translation of the description of *C. acremonium* Corda by Fresenius (17, p. 94-95):

On leaves of *Zea mais*, which became moist while being dried.

Delicate white mold. From creeping branched mycelium, which by strong magnification does not show septation plainly, arises, like the stalks in a spindle of a raceme, simple, nonseptate, conidiophores of practically equal length, which, at times plainly swollen at the base, gradually taper to a point like an awl and each bears a round head consisting of many spores held together by slime. These conidiophores develop either singly or crowded almost in whorls; they occur also with two terminal spore heads as in Figures 59 and 60; in the latter two very short projections can be seen which indicate that, in this case, both heads stood very close together. When placed in a drop of water, the spore heads dissolve immediately and the spores are dispersed. The spores vary considerably in size and form; they measure 1/300-1/170 mm. in length and, in outline, vary in shape from oval to oblong or almost linear (Fig. 4).

The general habit of the fungus strongly suggests that of *Acremonium*; except in the latter genus only a single spore is borne on the end of each conidiophore. The conidiophores with their spore heads are somewhat similar to *Hyalopus*.

Norton and Chen (34) reported a corn seed parasite in 1920 which they thought may have been described as *Oospora*, *Cephalosporium*, *Acrostalagmus*, or *Verticillium*. The occurrence of spores that are sometimes

septate and conform in size to the microconidia of Sheldon's *Fusarium moniliforme* (47) would differentiate it from *C. acremonium*.

#### ISOLATION OF ORGANISM AND GROWTH ON ARTIFICIAL MEDIA

The usual method of isolating the organism from the stalks was as follows: The stalk, while still turgid, was cut obliquely near the base to determine the presence of blackened bundles. If these bundles were present, pieces of the internodes were selected, split lengthwise, and immediately small portions of the blackened bundles were transferred aseptically to potato-agar slants or plates. Many of these gave rise to apparently pure cultures of *Cephalosporium acremonium* and, upon abundant sporulation, small pieces of the fungus were used to make spore suspensions from which dilution plates were poured. Transfers were made from single colonies to potato-agar slants. Internodes of the stalks are used because local infections by other organisms are somewhat common at the nodes, while organisms causing systemic infections are comparatively few in number. Hence, the different organisms to be found internally in the internodes are not abundant.

*Cephalosporium acremonium* grows on numerous culture media. It makes rapid progress on media containing a carbon compound, such as dextrose, maltose, lactose, saccharose, mannitol, or glycerine. Aerial mycelia with conidiophores and conidia usually are present on potato-dextrose agar and nutrient-dextrose agar at the end of three days' growth at temperatures between 17° and 32° C.

The hyphae in young cultures of recent isolations are in more or less loose cottony masses but appear somewhat coarse because of their tendency to agglutinate. The mycelium forms a moderately thick pale pink skin on the substrate by anastomosis and agglutination. It does not change the color of the agar. When the organism is first isolated it is usually pink or salmon colored and spore production is abundant. When carried for long periods on artificial media, it soon loses its color and more slowly it decreases in spore production and the quantity of aerial mycelia.

Some 3-year old isolations have no aerial mycelia except a few spikes of agglutinated hyphae which often form in rosettelike groups. Macroscopically, various isolations show some differences in culture, particularly in the intensity of the pink coloration and in the height and uniformity of the hyphae bearing the conidiophores. Isolations from sweet corn as a rule tend to be pinker in color and the little fascicles of aerial hyphae are more irregular and more curled than they are in isolations from dent corn. The spores are borne singly at the end of the conidiophore and collect in a mucilaginous ball containing from a few to as many as 50 or more. The fluid in which they are involved is copious enough to obscure any outline of the spores at the periphery of the sphere, giving them the appearance of glistening drops. When the spore head is placed in water the spores disperse immediately, but each spore retains a capsule. On account of this capsule, measurements of the spores vary with the moisture of the medium on which they are grown and with the manner in which they are mounted for examination. In India ink, allowed to dry at room temperature and examined immediately, they measure 3-6 by 1-1.8 microns and average 4.3 by 1.3 microns (Pl. 6, B, C, D). These measurements apply to isolations from both sweet and dent corn and also to the organism supplied by Manns and Adams.

Indicator media containing dextrose, saccharose, and glycerine, respectively, with cresol purple to detect increase in acidity and cresol red to detect increase in alkalinity were used in an attempt further to classify the various isolations of *Cephalosporium acremonium*. No data were obtained that would separate the sweet corn from the dent corn isolations. Although each isolation behaved consistently in a number of trials, it was found that two isolations from the same stalk might differ in their behavior on these media. Work is being continued on the organism, but for the present the various isolations, differing in minor characteristics, are regarded as various strains of *C. acremonium*. The organism supplied by Manns and Adams is also considered as representing this species rather than *C. sacchari*.

#### LIFE CYCLE OF CEPHALOSPORIUM ACREMONIUM

Infected ears carry the organism internally in the seed. As will be shown later, *Cephalosporium acremonium* is definitely pathogenic. It develops with the germinating kernel and causes a systemic infection of the plant through the vascular system (Pl. 6, A). By this means, it invades the ears and eventually the kernels. In this manner it is carried over to the following season. Occasionally an ear can be found which is externally overrun. In observations extending over a period of three years, only one such ear has been found. Probably an especially damp harvest season or poor storage conditions are necessary for this development. It seems possible that with such development, the infection might spread from ear to ear. Extensive inoculation experiments in which spore suspensions were placed on the seed gave, on the whole, negative results (see Table XI). However, the question of soil infection has not been determined definitely as yet.

Seed infection can be determined microscopically on limestone-sawdust germinators as described by Holbert and Hoffer (21, p. 14-16), but the symptoms are easily overlooked so that up to the present only a fair degree of accuracy has been attained by this method.

Infected germinating kernels have blanched, white tips, sometimes with noticeable mycelial growth. Often, however, the mycelial growth can not be seen until examined microscopically. When the symptoms on the germinator are overlooked the ears often are chosen for seed, because germination and the vigor of the infected seedlings are seldom impaired at this time.

In experiments, macroscopic examination of germinating kernels is followed by microscopic examination of those suspected. In this way the infected ears can be accurately determined (Pl. 6, E). In two instances 30 ears of this type were obtained. Two composites made from an equal number of kernels from each of these ears showed 65 per cent and 95 per cent *Cephalosporium* infections, respectively.

*Cephalosporium acremonium* has been isolated from black bundles in first leaves (Pl. 6, F), from bundles in the stalks (Pl. 1 (right), 3 B), from the shanks, from the cobs, and from the kernels.

#### INOCULATION EXPERIMENTS WITH CEPHALOSPORIUM ACREMONIUM

These experiments were conducted near Bloomington, Ill., on uniform, well-drained, fertile brown silt loam soil. The land on which the inoculation experiments were located never had been cropped previously.



TABLE XI.—Field and harvest data on the influence of seed inoculation with *Cephalosporium acremonium*. Seed inoculations were made at time of planting, May 11, 1921. Various strains of Yellow Dent corn grown on virgin, brown silt loam soil, Bloomington, Ill.

Plat No.	Field stand.				Suckers.		Purple stalks.		Prolific stalks.		Preharvest count of ears produced.		Barren stalks.		Sound marketable ears (by count after harvest).		Acre yield of shelled corn (corrected to uniform moisture basis).				Reduction in acre yields of inoculated plats.						
	Control.		Inoculated.		Control.		Inoculated.		Control.		Inoculated.		Control.		Inoculated.		Control.		Inoculated.		Control.		Inoculated.				
	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.			
1.	58	96.7	58	96.7	2	10	9	3	1	1	2	55	51	4	0	4	6	38	49	107.8	105.6	83.9	89.3	2.2	+6.4		
2.	57	95.0	57	95.0	10	10	5	3	6	4	5	11	6	5	5	11	15	30	28	95.4	87.9	68.7	57.6	7.5	+11.2		
3.	58	96.7	58	96.7	3	13	4	0	3	2	3	45	39	2	3	6	9	41	39	104.0	96.7	82.7	74.6	7.3	+9.8		
4.	53	88.3	53	88.3	19	20	1	0	4	4	4	43	41	6	4	9	15	30	25	88.9	99.7	66.7	53.9	+2.3	+12.8		
5.	57	95.0	57	95.0	13	13	4	0	4	2	4	48	47	4	2	9	10	39	37	98.6	99.7	76.3	71.6	+1.1	+4.7		
6.	56	93.3	56	93.3	10	10	2	2	4	2	4	52	46	2	4	9	7	35	34	98.3	96.7	69.6	66.5	1.8	+1.8		
7.	56	93.3	56	93.3	13	13	6	9	3	4	4	48	48	4	6	9	7	35	35	95.5	99.7	71.6	68.3	+4.2	+4.5		
8.	57	95.0	57	95.0	16	16	1	4	5	2	5	50	43	2	5	4	11	33	36	93.2	99.7	63.9	74.2	+6.5	+16.1		
9.	56	93.3	56	93.3	13	13	1	0	4	5	4	44	42	9	3	10	4	34	37	87.0	90.8	57.9	49.5	+9.7	+2.9		
10.	57	95.0	57	95.0	15	15	1	5	3	3	51	44	3	6	7	10	7	31	27	97.2	77.9	72.2	74.9	+3.3	+8.8		
11.	58	96.7	58	96.7	22	22	1	5	4	4	42	39	7	8	10	12	28	29	81.4	82.0	51.9	51.7	+0.6	+0.2			
12.	57	95.0	57	95.0	12	12	3	1	4	2	4	45	40	4	6	6	7	32	35	73.5	86.0	62.7	66.8	+7.4	+11.7		
13.	58	96.7	58	96.7	7	7	2	4	2	4	47	46	4	2	14	12	33	32	94.7	82.0	53.9	59.8	9.8	12.5			
14.	56	93.3	56	93.3	8	8	1	4	1	2	4	40	41	4	8	9	11	40	40	73.8	84.9	44.1	42.8	+1.1	2.9		
15.	57	95.0	57	95.0	5	5	2	4	3	3	40	42	7	4	9	11	28	22	74.6	72.4	55.9	72.4	+9.6	+29.5			
16.	54	90.0	54	90.0	6	6	5	6	3	4	3	38	39	9	2	11	14	32	26	78.7	74.6	48.9	42.8	+	12.5		
17.	58	96.7	58	96.7	4	4	3	1	7	4	7	46	41	7	6	9	7	23	27	72.0	79.4	41.6	49.8	+4.1	+19.4		
18.	56	93.3	56	93.3	5	5	6	3	4	1	3	42	39	5	9	14	7	23	27	72.0	79.4	41.6	49.8	+7.4	+19.7		
19.	54	90.0	54	90.0	5	5	6	4	2	2	3	42	39	5	9	14	7	23	27	72.0	79.4	41.6	49.8	+7.4	+19.7		
20.	54	90.0	54	90.0	5	5	6	4	2	2	3	42	39	5	9	14	7	23	27	72.0	79.4	41.6	49.8	+7.4	+19.7		
Total.	1,134	.....	1,119	.....	204	213	75	36	45	70	917	879	96	106	179	188	648	657	1,758.3	1,774.1	1,268.7	1,242.6	.....	.....	.....	.....	
Per cent increase following inoculation.	.....	.....	.....	.....	.....	4.4	.....	.....	55.6	.....	.....	.....	.....	.....	10.4	.....	5.03	.....	1.4	.....	0.9	.....	.....	.....	.....	.....	.....
Per cent decrease following inoculation.	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
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TABLE XII.—Data showing comparative field stand, number of suckers, barren stalks, and total and marketable ears, and yields of shelled corn from various strains of Yellow Dent corn, inoculated hypodermically when about 12 inches high with *Cephalosporium acremonium* and from controls injected with corresponding amounts of sterile water, planted May 11, 1921, in adjacent plats on uniform virgin brown silt loam soil, near Bloomington, Ill.

Flat No.	Final field stand.				Suckers.		Purple stalks.		Prolific stalks.		Preharvest count of ears produced.				Barren stalks.		Sound, marketable ears (by count after harvest.)		Acre yield of shelled corn (corrected to uniform moisture basis).					
	Control.		Inoculated.		Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Good ears.		Nubbin ears.		Control.	Inoculated.	Control.	Inoculated.	Control.	Inoculated.	Total yield.		Yield of marketable corn.	
	Num-ber.	Per cent.	Num-ber.	Per cent.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Num-ber.	Bush-els.	Bush-els.	Bush-els.	Bush-els.	Bush-els.	Bush-els.
1.....	56	93.3	57	95.0	19	32	0	0	1	4	53	44	6	6	10	40	33	106.0	91.6	77.5	69.4	77.5	69.4	77.5
2.....	58	96.7	57	93.3	6	37	0	1	3	3	45	37	7	7	11	15	20	98.9	77.2	63.9	47.3	63.9	47.3	
3.....	59	98.3	57	93.3	14	51	0	2	0	2	56	39	1	8	5	14	24	102.3	85.7	81.2	59.8	81.2	59.8	
4.....	56	91.3	56	93.3	18	44	1	0	3	7	43	35	7	12	9	15	19	89.5	75.7	62.9	41.0	62.9	41.0	
5.....	59	98.3	56	93.3	13	21	0	0	0	6	51	45	2	3	7	9	42	104.1	86.0	84.9	64.6	84.9	64.6	
6.....	57	95.0	59	98.3	16	29	2	5	1	2	54	45	2	6	6	8	31	97.8	92.3	56.5	63.1	92.3	56.5	
7.....	58	96.7	59	98.3	22	35	0	1	0	4	49	47	7	9	6	9	39	93.7	89.0	78.3	61.3	89.0	78.3	
8.....	57	95.0	59	98.3	11	26	0	1	0	3	54	41	1	5	9	13	35	90.1	83.8	65.3	54.6	83.8	65.3	
9.....	59	98.3	57	95.0	37	38	0	0	3	7	46	37	5	7	9	13	35	90.1	83.8	65.3	54.6	83.8	65.3	
10.....	58	96.7	59	98.3	7	38	0	2	0	5	46	37	11	15	7	16	31	93.8	84.5	73.5	64.2	84.5	73.5	
11.....	59	98.3	52	86.7	15	48	1	1	4	4	50	38	2	8	9	12	38	95.6	84.5	73.5	64.2	84.5	73.5	
12.....	56	93.3	54	90.0	21	50	2	1	3	3	38	29	6	8	16	20	25	92.3	73.8	69.4	52.0	73.8	69.4	
13.....	59	98.3	54	90.0	16	38	2	3	5	5	48	36	2	2	10	18	36	78.3	73.8	69.4	52.0	73.8	69.4	
14.....	53	88.3	60	100.0	10	39	1	2	1	1	41	43	7	7	6	12	31	110.8	79.7	90.4	57.2	79.7	90.4	
15.....	59	98.3	58	96.7	8	36	5	12	4	7	45	46	7	8	9	12	36	72.0	65.3	43.2	41.0	65.3	43.2	
16.....	59	98.3	58	96.7	20	28	3	3	3	8	43	36	7	8	9	16	30	76.8	65.3	52.1	35.1	65.3	52.1	
17.....	59	98.3	59	98.3	10	28	2	3	5	8	32	25	13	15	14	19	25	16	65.3	57.2	41.7	30.3	65.3	57.2
18.....	57	95.0	57	95.0	23	25	5	5	3	6	45	30	6	13	12	16	34	19	86.1	66.9	59.8	35.1	86.1	66.9
19.....	59	98.3	53	88.3	24	37	5	10	1	7	42	27	7	11	9	19	26	17	72.0	55.4	47.6	38.4	72.0	55.4
20.....	54	90.0	55	91.7	8	20	1	2	2	5	35	39	10	6	11	12	14	28	70.0	57.9	47.6	38.4	70.0	57.9
21.....	51	85.0	54	90.0	11	42	3	5	1	5	35	39	10	6	11	12	14	28	70.0	57.9	47.6	38.4	70.0	57.9
22.....	53	88.3	57	95.0	16	48	2	7	2	1	42	40	7	17	7	13	34	31	62.4	75.3	60.2	53.9	62.4	75.3
23.....	48	80.0	53	88.3	3	29	2	8	6	7	34	34	8	6	7	16	26	18	70.5	62.0	51.7	35.8	70.5	62.0
24.....	51	85.0	60	100.0	21	52	0	5	3	3	42	42	1	7	9	14	30	84.9	83.1	54.6	41.0	84.9	83.1	
25.....	51	85.0	46	76.7	10	26	3	2	2	6	34	34	6	10	8	12	9	19	70.5	65.3	39.1	28.1	70.5	65.3
26.....	51	85.0	50	83.3	13	53	7	1	1	2	37	38	1	8	11	12	28	25	76.8	70.9	55.4	43.9	76.8	70.9
27.....	56	93.3	55	91.7	21	22	3	2	1	2	45	39	4	4	10	13	21	25	79.4	83.4	42.5	53.5	79.4	83.4
28.....	53	86.7	55	91.7	21	22	3	2	1	2	45	39	4	4	10	13	21	25	79.4	83.4	42.5	53.5	79.4	83.4
Mean.....	56.1	93.6	55.9	93.1	14.5	35.5	2.0	3.1	2.1	4.5	44.0	37.5	5.9	8.4	8.9	13.8	25.1	84.8	75.1	59.6	47.7	75.1	59.6	47.7



The plats were hand-planted in hills 42 inches apart each way at the rate of 3 kernels per hill. Great care was exercised throughout the season to avoid mechanical injury of the plants during cultivation, and to guard against insect pests and rodents, but no attempt was made either to thin to a uniform stand or to correct for differences in stand.

Acre yields have been reduced to a uniform moisture basis of 14 per cent and reported as "Total yield" and "Yield of marketable corn," the latter obtained from the former by excluding small nubbins, rotten ears, and light chaffy ears as described by Holbert et al (22).

Table XI gives the field and harvest data on the influence of inoculating various strains of Yellow Dent corn with *Cephalosporium acremonium* isolated from dent corn. The inoculum was in the form of a spore suspension in sterile water, enough spores from potato-dextrose agar cultures being used to produce a moderate cloudiness in the water. The seed was soaked in the inoculum not to exceed one-half hour and then planted.

As was noted above under life cycle of the organism, Table XI is a presentation of data on seed inoculation. The effects in this case are almost negative.

Table XII gives the results of inoculations with *Cephalosporium acremonium* with all conditions similar to those in Table XI except that the inoculations were made by means of a hypodermic syringe when the plants were about 12 inches in height. Approximately 0.2 cc. of the suspension of the organism was injected into the central portion of each stalk at about 2 inches above the crown of the plant. Control plants were injected with corresponding amounts of sterile water. The difference in final field stands of the inoculated plats and the control plats averaged less than one-half per cent.

TABLE XIII.—Summary of data presented in Table XII

Points of comparison.	Control.	Inoculated.	Per cent increase following inoculation.	Per cent decrease following inoculation.	Odds.
Mean percentage final field stand.....	93.6	93.1	.....	0.5	.....
Mean number of suckers per plat.....	14.5	35.5	144.8	.....	> 999:1
Mean number of purple stalks per plat..	2.0	3.1	55.0	.....	74:1
Mean number of prolific stalks per plat.	2.1	4.5	114.3	.....	> 999:1
Mean number of barren stalks per plat..	8.9	13.8	55.1	.....	> 999:1
Mean number of nubbins ears per plat...	5.9	8.4	42.4	.....	> 999:1
Mean total yield (bushel) per plat.....	84.8	75.1	.....	11.4	> 999:1
Mean yield of marketable corn (bushel) per plat.....	59.6	47.7	.....	20.0	> 999:1

It will be noted from Table XIII, which gives a summary of the data in Table XII, that there was a 144.8 per cent increase in number of suckers, 55 per cent increase in purple stalks, 114.3 per cent increase in prolific stalks, 55.1 per cent increase in number of barren stalks, 42.4 per cent increase in number of nubbins, 11.4 per cent decrease in total yield, and 20.0 per cent decrease in yield of marketable corn in the plats inoculated with *Cephalosporium acremonium*. The increase in the number of suckers on the inoculated plants was the most apparent symptom in the plats. This

also was the case in 1922 both in inoculated plants from horny, nearly disease-free seed (Pl. 5 A, B, C) and in ear rows from naturally infected seed (Pl. 4 A, B). However, inoculations of various strains of corn with this organism do not produce this symptom consistently. Starchy composites, especially, show less tendency to sucker when inoculated with this organism, but the main stalks are more markedly affected, resulting in numerous weak, spindly, low-yielding plants (Pl. 5 C, D). In this latter case the reduction in yield generally is greater than in better strains of corn which show increase of suckering.

Table XIV gives the results of inoculations with *Cephalosporium acremonium* with all conditions similar to those described in Table XII, except that the date of planting, May 28, was later, that the control plants were not injected with sterile water, and that five rows in a third series were inoculated with *Aplanobacter stewarti* (E F S) McCul. This bacterial organism, also pathogenic on dent corn, is further similar to *C. acremonium* in its attack on corn in that it causes vascular infections of the plants. Yields of the plats, the plants of which were injected with sterile water, were compared with the yield from a number of corresponding control plats not thus injected. The injections of water seemed to have no effect. In every case the yields from the group of plants receiving sterile water injections equaled or slightly exceeded the corresponding ones not thus injected. Therefore, mechanical injury caused by the inoculations was considered negligible.

In agreement with the results given in Tables XII and XIII, the results given in Tables XIV and XV show an increase of suckers, purple stalks, prolific stalks, nubbin ears, and barren stalks, and a significant decrease in good ears and yield from the plants inoculated with *Cephalosporium acremonium*.

Comparing the inoculations of the two parasites, *Cephalosporium acremonium* increased the number of purple stalks (152.4 per cent) while *Aplanobacter stewarti* did not increase them, and although *Aplanobacter stewarti* increased suckering, the *C. acremonium* plats averaged 110.8 per cent more suckers than the *A. stewarti* plat. In a similar way there were 55.3 per cent more nubbin ears in the *C. acremonium* plats than in the *A. stewarti* plats. The number of barren stalks was increased and the yields decreased about the same amount in both inoculations. Therefore, the outstanding differences in the effects of those two pathogens causing vascular diseases of corn are the increase in the number of purple stalks and the larger number of suckers, prolific stalks, and nubbin ears caused by *C. acremonium*.

In Tables XII to XV it will be noted that the reductions in yield due to the hypodermic inoculations with *Cephalosporium acremonium* and *Aplanobacter stewarti* range from slight increases to as much as 44.6 per cent reduction in acre yields of marketable corn. Table XVI presents data comparing the resistance and susceptibility of different seed selections from various strains of Yellow Dent corn to the vascular diseases induced by *C. acremonium* and *A. stewarti*. Data from inoculations with *Gibberella saubinetii* on corn grown from similar seed from the same sources are included to show that, in general, seed lots susceptible to these two vascular diseases are likewise susceptible to one of the rootrot organisms.

TABLE XIV.—Data showing comparative field stand, number of suckers, barren stalks, and total and marketable ears, and yields of shelled corn from various strains of Yellow Dent corn uninoculated and inoculated hypodermically when about 12 inches high with *Aplanobacter stewartii* and *Cephalosporium acremonium*, respectively, planted May 28, 1921, in adjacent plots on uniform, virgin, brown silt loam soil, at Bloomington, Ill.

Plat No.	Final field stand (60 plants perfect stand).						Suckers.			Purple stalks.			Profile stalks.			Preharvest count of ears produced.				Barren stalks.				Sound marketable ears (by count after harvest).				Acre yield of shelled corn (corrected to uniform moisture basis).																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
	Control.			A. stewartii.			C. acremonium.			Control.			A. stewartii.			C. acremonium.			Good ears.		Nubbin ears.		Control.		A. stewartii.		C. acremonium.		Control.		A. stewartii.		C. acremonium.		Control.		A. stewartii.		C. acremonium.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																	
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1.	60	100.0	59	98.3	59	98.3	6	10	16	1	2	4	0	0	5	8	10	2	8	5	1	7	8	36	91.9	84.9	90.1	70.5	63.7	64.2	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	63.7	

TABLE XV.—Summary of data presented in Table XIV

Points of Comparison.	Control.	Inoculated.		Increase or decrease following inoculation.			
		<i>A. stewarti.</i>	<i>C. acremonium.</i>	<i>A. stewarti.</i>		<i>C. acremonium.</i>	
				<i>Per cent.</i>	<i>Odds.</i>	<i>Per cent.</i>	<i>Odds.</i>
Mean percentage final field stand.....	97.9	97.5	96.0	0.4	.....	1.9	.....
Mean number of suckers per plat.....	5.0	15.8	33.3	216.0	>999:1	566.0	>999:1
Mean number of purple stalks per plat.....	2.1	2.1	5.3	.....	.....	152.4	>999:1
Mean number of prolific stalks per plat.....	1.6	2.1	3.3	31.3	15:1	106.3	195:1
Mean number of barren stalks per plat.....	7.4	9.6	9.9	29.7	554:1	33.8	356:1
Mean number of nubbin ears per plat.....	2.6	3.8	5.9	46.2	78:1	126.9	>999:1
Mean total acre yield (bushel) per plat.....	90.0	84.8	85.5	-5.8	554:1	-5.0	199:1
Mean acre yield of marketable corn (bushel) per plat.	68.6	59.1	60.7	-13.8	>999:1	-11.5	>999:1

The nearly disease-free control (Table XVI) was a composite of seed ears selected from apparently healthy plants and showing no evidence of rotting or infection when tested on a limestone-sawdust table germinator. This strain of Yellow Dent corn is the result of several years careful plant and germinator selection to avoid seed from plants affected with any of the root, stalk, and ear-rot diseases. Corn grown from this seed was highly resistant to injury following inoculation with *Gibberella saubinetii*, but was damaged to the extent of 12.9 per cent by inoculation with *Cephalosporium acremonium*. Although care had been exercised to avoid plants showing purple leaves and multiple ears, it is probable that many ears were included from year to year that had grown on plants more or less infected with *C. acremonium*. This fact may account for the greater susceptibility of this corn to *C. acremonium* than to *G. saubinetii*. It is also interesting to note that the nearly disease-free control corn was not equally susceptible to the two vascular diseases induced by *C. acremonium* and *A. stewarti*, respectively, being rather susceptible to the former, but highly resistant to the latter.

The other lots of seed referred to in Table XVI were furnished by three representative farmers from three different counties in central Illinois. From these lots of seed, two selections of approximately 300 ears each were chosen on the basis of physical appearance. Ears included in the "good seed" selection had been grown on plants apparently not much affected by any of the root, stalk, and ear-rot diseases, while the ears included in the "susceptible seed" selection bore evidence of having been produced on diseased plants. No dead or weak-germinating ears were included. Data presented in Table XVI show that the corn grown from the susceptible seed selection was affected to a much greater extent, as expressed in terms of acre yield, by the inoculations with both *C. acremonium* and *A. stewarti* than was the corn grown from the good seed selection. Corn grown from the susceptible seed selection also was more susceptible to injury following inoculation with *G. saubinetii*.

TABLE XVI.—Yield data from certain selections and strains of Yellow Dent corn when inoculated with *Cephalosporium acremonium*, *Aplanobacter stewartii*, and *Gibberella saubinetii*, the corn being grown near Bloomington, Ill., in 1921, on virgin brown silt loam soil of high fertility

Character of seed.	Inoculated with—	Number of plats.	Total acre yield.		Reduction following inoculation.		Odds.
			Control.	Inoculated.			
			Bushels.	Bushels.	Bushels.	Per. ct.	
Susceptible selection...	<i>C. acremonium</i> ...	6	84.0	65.7	18.3	21.8	>999:1
Good seed selection.....	do.....	6	88.0	81.5	6.5	7.4	45:1
Susceptible selection...	<i>A. stewartii</i> .....	6	85.6	74.1	11.5	13.4	59:1
Good seed selection.....	do.....	6	84.1	85.0	+0.9	+1.1	1:1
Susceptible selection...	<i>G. saubinetii</i> .....	6	84.6	69.0	15.6	18.4	158:1
Good seed selection.....	do.....	6	85.7	80.6	5.1	6.0	8:1
Nearly disease-free control.....	<i>C. acremonium</i> ...	14	89.4	77.9	11.5	12.9	>999:1
Nearly disease-free control.....	<i>A. stewartii</i> .....	14	93.9	91.1	2.8	3.0	37:1
Nearly disease-free control.....	<i>G. saubinetii</i> .....	14	90.4	87.5	2.9	3.2	50:1

A further study of the field performance of these same seed lots is given in Table XVII. Corn grown from the susceptible seed selection, which was injured to the extent of 21.8 per cent, 13.4 per cent, and 18.4 per cent by inoculations with pure cultures of *C. acremonium*, *A. stewartii*, and *G. saubinetii*, respectively, yielded  $8.6 \pm 1.1$  bushels less sound corn than corn grown from the good seed selection which was highly resistant to injury from inoculations with all three organisms.

TABLE XVII.—Yield data from the susceptible and good selections and the nearly disease-free control, grown near Bloomington, Ill., in 1921, on infested brown silt loam soil of medium fertility

Character of seed.	Number of plats.	Acre yield.		Increase in yield of marketable corn of good seed selection over susceptible selection.			Increase in yield of marketable corn of nearly disease-free check over good seed selection.		
		Total.	Marketable.						
		Bushels.	Bushels.	Bushels.	Per cent.	Diff. P. E.	Bushels.	Per cent.	Diff. P. E.
Susceptible seed selection....	42	74.8 ± 0.5	46.5 ± 0.7	8.6 ± 1.1	18.5	7.8	.....	.....	.....
Good seed selection.....	42	79.6 ± 0.7	55.1 ± 0.8	.....	.....	.....	.....	.....	.....
Nearly disease-free check....	42	86.4 ± 0.5	64.3 ± 0.5	.....	.....	.....	9.2 ± 0.9	16.7	10.2

Additional data on the field performance of the susceptible and good seed selections at various points in Illinois are presented in Table XVIII. Although the difference in yield between these two selections was not large at Bloomington, nor at Peoria, where corn followed clover, yet the mean difference in yield at all points was 11.9 bushels, or 15.4 per cent, with odds of 58:1. The mean difference in yield of marketable corn was larger.

Since these data (Tables XVI, XVII, XVIII) show that resistance to the two vascular diseases (Table XVI) probably is closely associated with the ability to yield well in soil infested with disease-producing

organisms and under unfavorable environment, it is thought that plant breeders may take advantage of this relationship in developing productive strains and varieties of corn more resistant to root, stalk, and ear-rot diseases. Eliminations of homozygous strains on the basis of susceptibility could be made by inoculating with one or both of the vascular disease organisms. The authors also feel that inoculations with *Cephalosporium acremonium* and *Aplanobacter stewarti* would add considerably to the value of corn varietal tests.

TABLE XVIII.—Yield data of corn grown from good seed and susceptible seed selections, the experiments being conducted on brown silt loam soil at various points in Illinois, in 1921

Location (Illinois).	Previous crop.	Total acre yield.		Decrease in yield from susceptible seed selection.
		Good seed selection.	Susceptible seed selection.	
		Bu.	Bu.	Bu.
Bloomington.....	Virgin prairie sod.....	88.9	84.5	4.4
Do.....	Corn.....	78.4	74.6	3.8
Do.....	do.....	83.3	75.8	7.5
Peoria.....	Clover.....	73.4	71.5	1.9
Do.....	Corn.....	81.5	66.6	14.9
Do.....	do.....	83.2	66.7	16.5
Do.....	do.....	85.4	62.8	22.6
Virginia.....	Clover.....	69.8	50.1	19.7
Do.....	do.....	67.0	59.5	7.5
Do.....	do.....	64.4	43.8	20.6
Mean.....		77.5	65.6	<sup>a</sup> 11.9

<sup>a</sup> Odds 58:1.

### SUMMARY

This paper presents the results of investigations of a hitherto unreported disease of corn and of an organism capable of producing it.

The most distinguishing symptom of this disease is the presence of blackened vascular bundles in the stalks and sometimes in the leaves. Associated with the disease to a notable extent are the following abnormalities: Excessive sucker production, prolific stalks, manifestations of which are a tendency for ear development at many nodes or multiple-ear production at one node; a certain type of reddening or purpling of the leaves and stalks; stalks with aborted ears (barren); and stalks bearing nubbins only.

The first part of this paper deals with this group of symptoms in a general way without special regard to causes. The data presented in this connection are based mainly on leaf and stalk color manifestations. However, it was noted that the other characteristics of this group were closely associated.

The data on economic importance show that high percentages of diseased stalks (46.6 per cent in the untreated seed and 42.7 per cent in the treated seed), result from planting ears selected from purple stalks and that yields are reduced. The yield data are presented in two ways. The first shows the average reduction in yield of purple-stalked plants (33.4 per cent with untreated seed and 20.6 per cent with treated seed). This

might be used in estimating loss whenever survey data are at hand. The second shows the reduction in yield due to selecting seed ears from purple-stalked plants (19.7 per cent), and, therefore, the advisability of avoiding selection of seed ears from stalks exhibiting this symptom.

*Cephalosporium acremonium* has been found closely associated with this group of symptoms and has produced many of these symptoms by pure culture inoculations. Another symptom brought out more strongly by artificial inoculations is an increase in suckering.

The disease caused by this organism is seed-borne and vascular. Therefore, when experimenting with this particular organism, the blackened fibro-vascular bundles were considered the most distinguishing characteristic. Purpling, prolific stalks, barren stalks, nubbin ears, and suckering were noted as being closely associated.

The organism enters the seed through the vascular system and lives within it until placed under conditions favorable for germination. Generally it does not inhibit germination or affect the early vigor. It specifically affects grain production and a general blighting sets in near the end of the growing season.

In one inoculation experiment comprising more than 3,000 plants of Yellow Dent corn and designed to give reliable yield data, the half inoculated with a pure culture of *Cephalosporium acremonium* produced 11.4 per cent less in total yield and 20.0 per cent less in marketable corn than the other half used as a control. The inoculated corn showed also increases of 144.8 per cent in number of suckers, 55 per cent in purple stalks, 114.3 per cent in prolific stalks, 42.4 per cent in nubbin ears, and 55.1 per cent in barren stalks.

Inoculations of a number of strains of Yellow Dent corn have shown different degrees of susceptibility. Even very susceptible strains of this corn may react differently to invasions of this organism as reflected in their outward characteristics.

To reduce losses from this disease it is well to avoid selection of seed ears from stalks having any of this group of symptoms. Probably the best measure of control will come with the development of resistant strains of corn within the varieties. Preliminary seed treatment experiments, not yet ready to be reported in this paper, offer promise of control of this disease.

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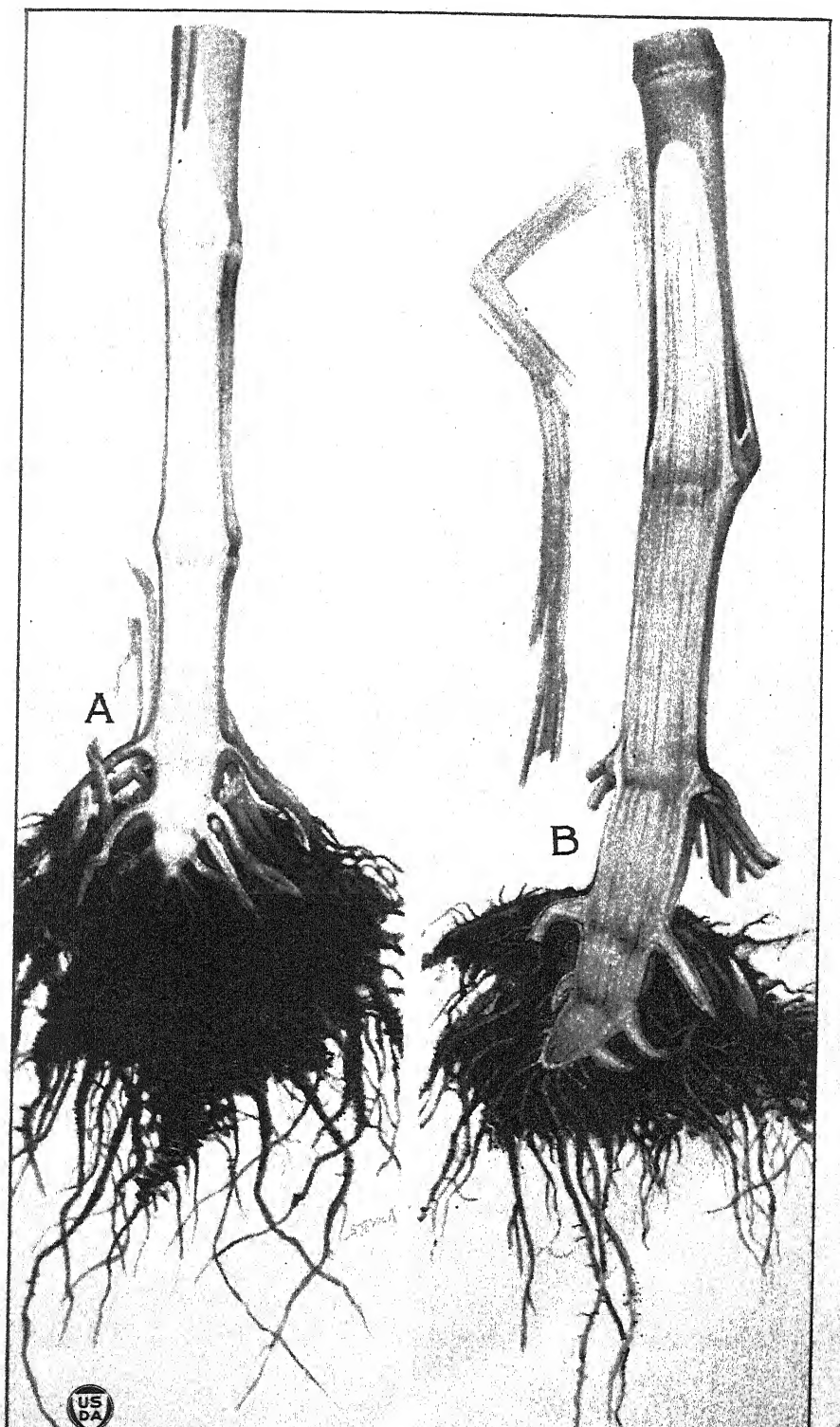


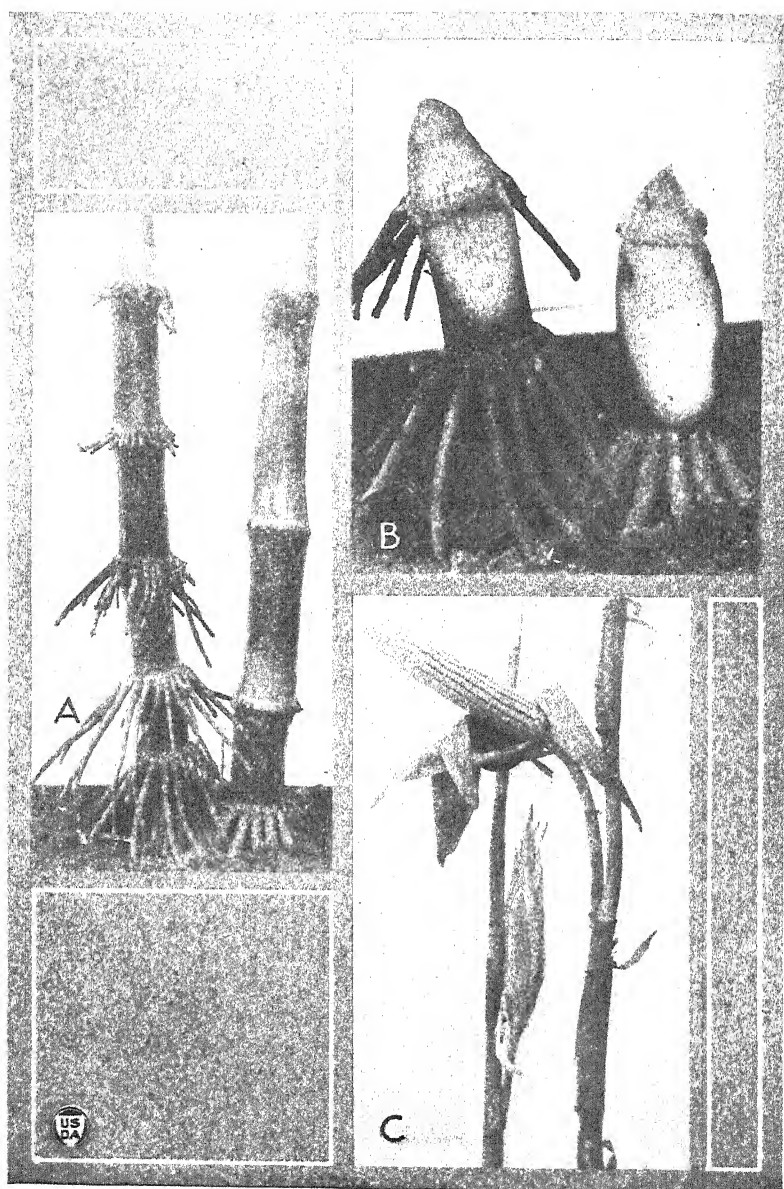
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PLATE 1

Bases of Yellow Dent corn plants at time of harvest cut to show internal condition. Healthy plant at left; plant at right infected with *C. acremonium*, showing characteristic blackened bundles and purple stalk.

(206)





## PLATE 2

Portions of diseased (left) and healthy (right) Yellow Dent corn plants in the same hill, grown at Bloomington, Ill., 1918.

A.—Basal portions showing excessive brace roots on diseased stalk (left).

B.—Oblique sections through the basal portions of same stalks. The vascular bundles of the diseased plant (left) are black, while those of the healthy plant are normal in color.

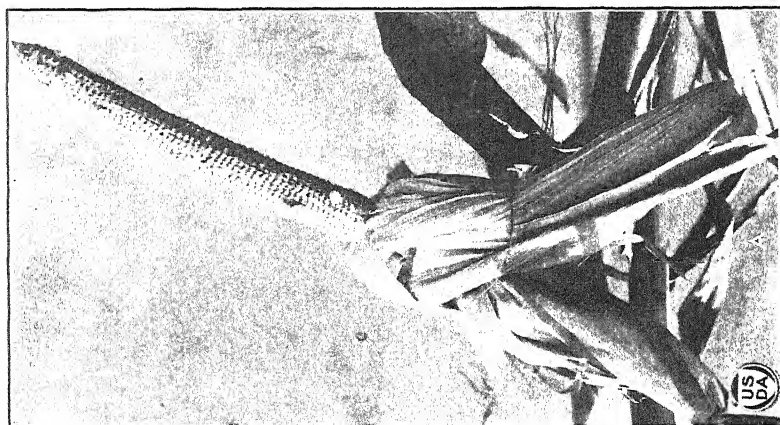
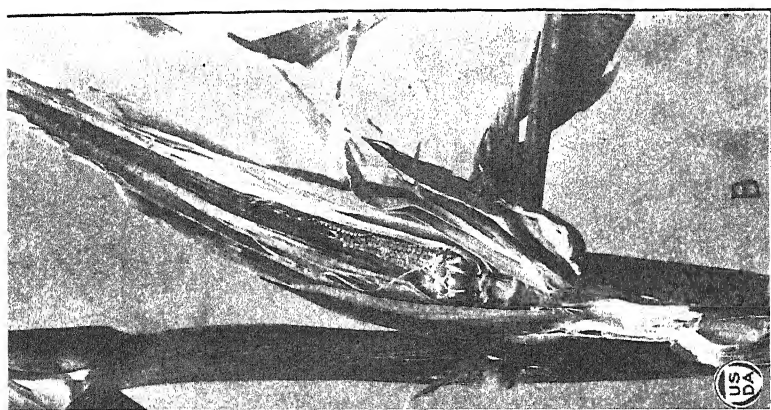
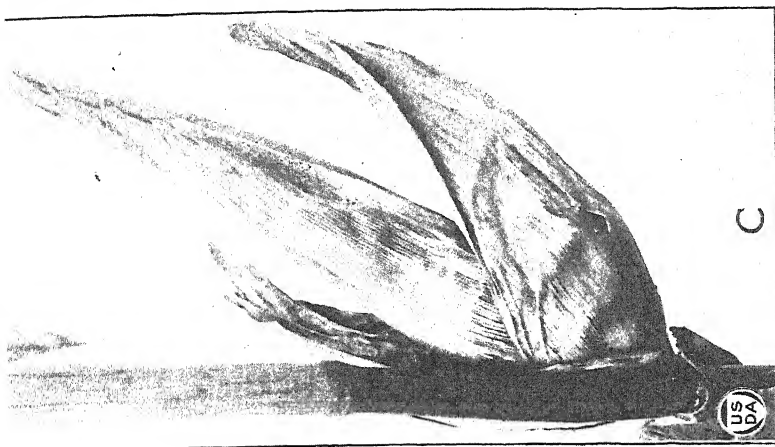
C.—The ear bearing portions of the same two stalks. Diseased stalk (left) with aborted ear; healthy stalk (right) with healthy ear.

### PLATE 3

A.—Portion of a main stalk of Funk Ninety-Day corn (*Zea mays indentata*) showing one type of barrenness. The cob is of normal length but the grain production is practically nothing. The leaves were purpled and many of the vascular bundles in the stalk were almost black. Bloomington, Ill., 1920.

B.—Portion of a main stalk of corn (*Zea mays indentata*) showing an extreme case of the most common type of barrenness found in connection with the black-bundle disease of corn. The ear is almost entirely aborted. In other typical cases small nubbins of various lengths are produced which usually curve toward the stalk. Bloomington, Ill., 1920.

C.—Portion of a main stalk of corn (*Zea mays indentata*) showing an attempt at multiple ear production. A high percentage of such plants have blackened vascular bundles in the stalks. Bloomington, Ill., 1920.



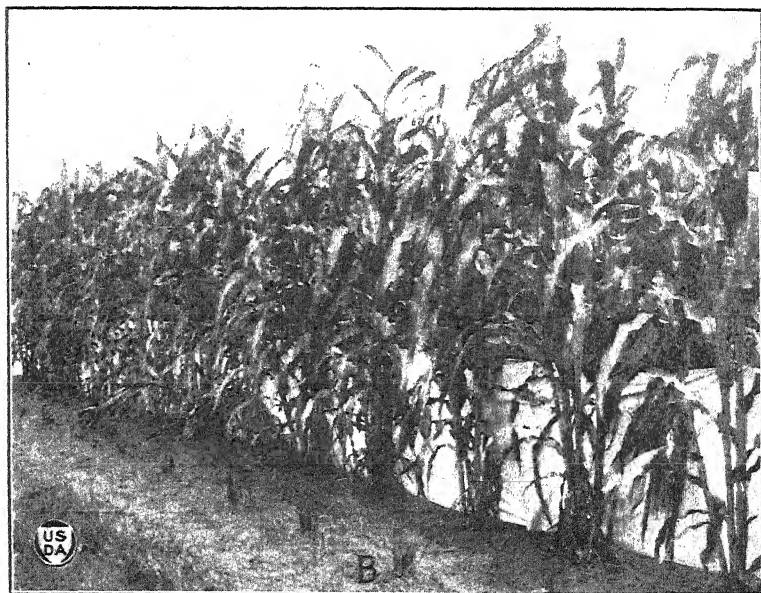
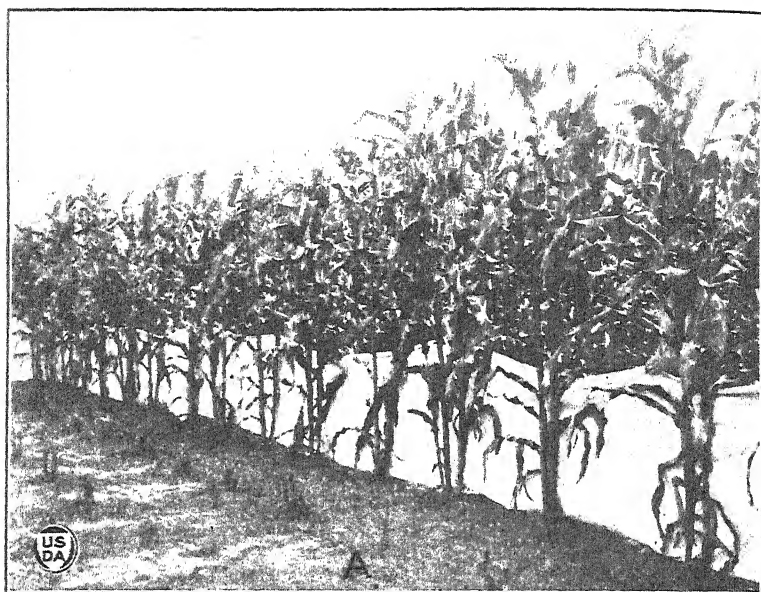




PLATE 4

Ear rows of Yellow Dent corn planted early on brown silt loam soil of high fertility, near Bloomington, Ill., and photographed in August, 1922.

A.—Grown from nearly disease-free seed. Note absence of excessive suckering.

B.—Grown from the same strain of corn as in A but naturally infected with the fungus, *C. acremonium*. Note excessive suckering.

## PLATE 5

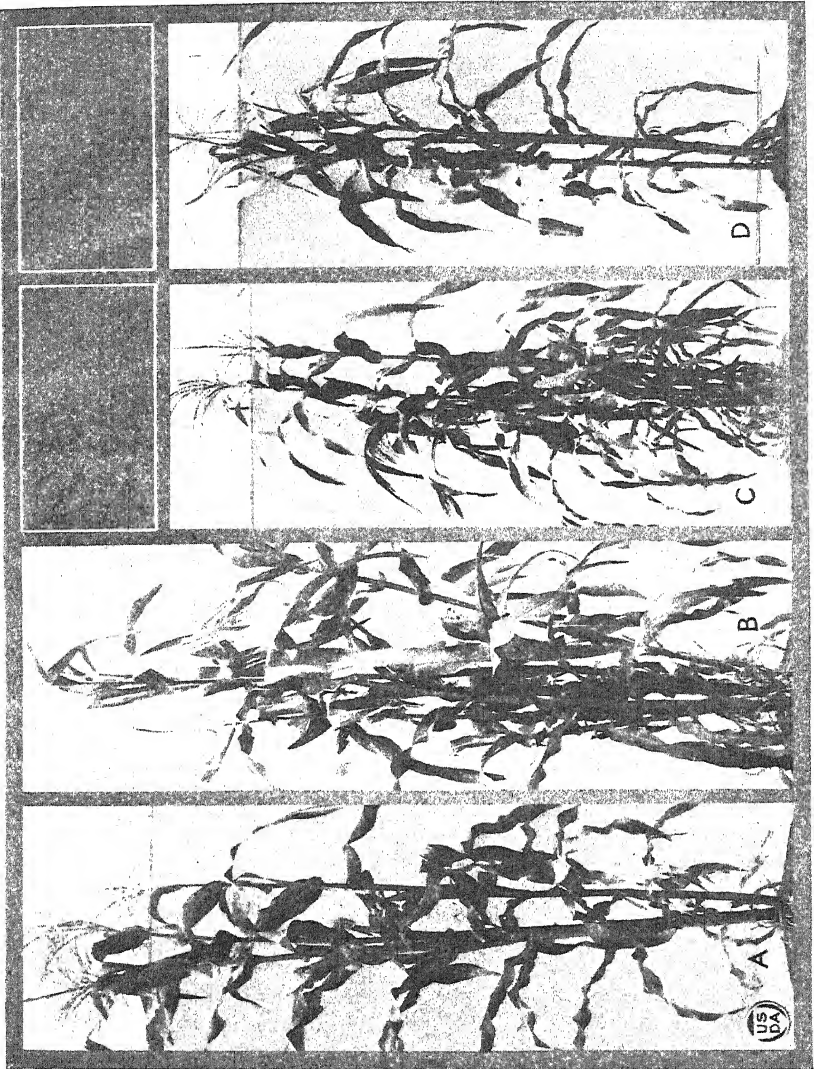
Hills of Yellow Dent corn, uninoculated and inoculated with *C. acremonium*.  
Bloomington, Ill., 1922.

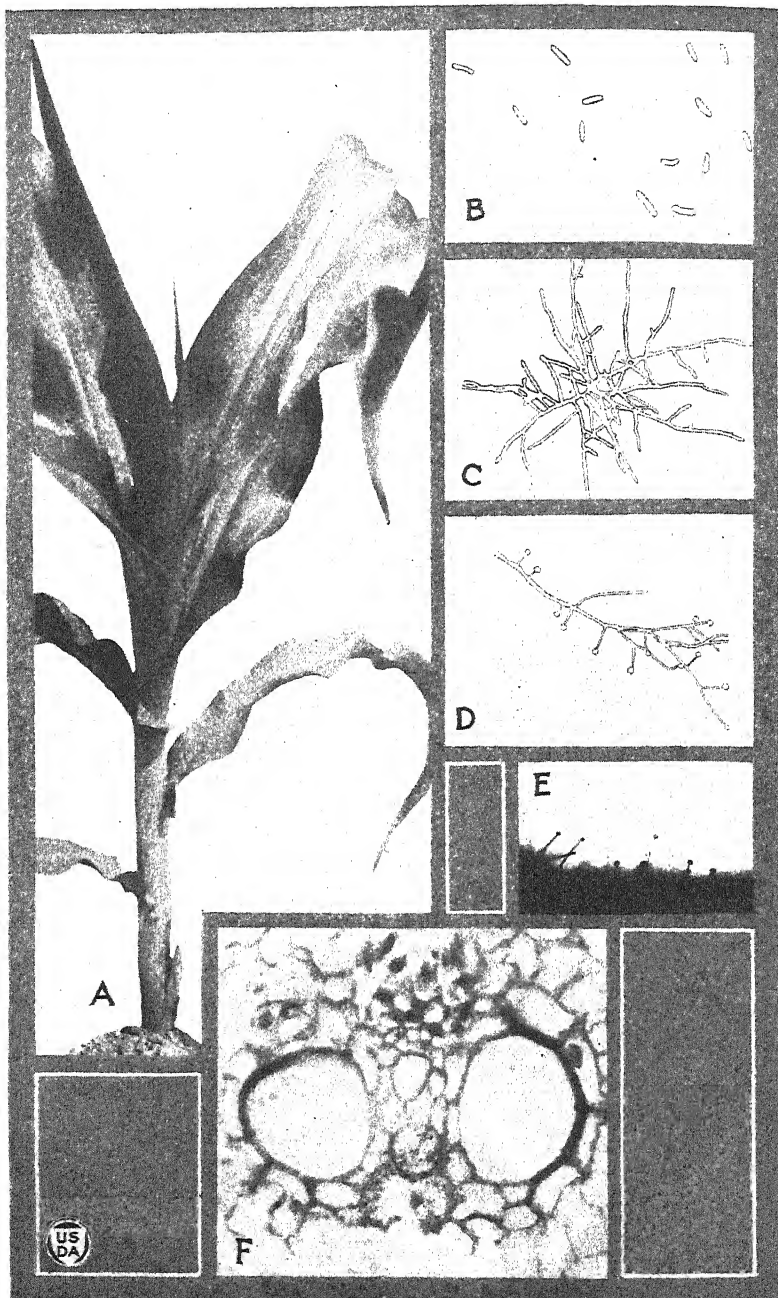
A.—Hill of corn in the uninoculated control plat grown from nearly disease-free seed. Note the absence of suckering, together with normal formation of ears.

B.—Hill of corn in the inoculation plat (inoculated with *C. acremonium*) grown from nearly disease-free seed. Note the number of suckers.

C.—Hill of corn from nearly disease-free, horny seed; plants inoculated with *C. acremonium*. Note normal development of stalks and ear shoots but an excessive number of suckers.

D.—Hill of corn from weak, starchy seed; plants inoculated with *C. acremonium*. Note spindly stalks without suckers or ear shoots.





## PLATE 6

A.—Portion of corn plant about three weeks old showing stripes in the leaves caused by natural infection of some of the bundles with *C. acremonium*.

B.—Spores of *C. acremonium*. ( $\times 1000$ )

C.—Spore of *C. acremonium* 44 hours after germination on potato dextrose agar. ( $\times 250$ )

D.—Piece of mycelium of *C. acremonium*. ( $\times 200$ )

E.—*Cephalosporium acremonium* as it appears on the funiculus of germinating kernels of corn when mounted under the low power of the compound microscope. ( $\times 200$ )

F.—Photomicrograph of a cross section of a blackened vascular bundle in a green leaf of a young dent corn plant. The mycelium of *C. acremonium* is shown almost completely filling the spiral and the annular vessel and occasional strands are shown in the small pitted vessels between the two large pitted vessels.



# CHANGES IN HYDROGEN-ION CONCENTRATION PRODUCED BY GROWING SEEDLINGS IN ACID SOLUTIONS<sup>1</sup>

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## INTRODUCTION

The importance of the reaction (the active acidity or alkalinity) of the medium in plant life, as well as in biological processes in general, has long been recognized. Studies in this field, however, have gained an impetus with the introduction of improved and delicate methods for estimating hydrogen-ion concentration.

Taking advantage of the ease of manipulation which characterizes these methods, agricultural investigators and botanists have secured a great many data on the relation between plants and the reaction of the medium in which they grow. Much of the recent work, undertaken for the purpose of establishing fundamental principles, has been conducted with nutrient solutions of known composition and with seedlings under conditions involving the least interference from unknown disturbing factors.

Most of the investigations thus far reported have dealt primarily with the effect of the reaction of the medium on plant growth, their ultimate goal being the determination of the specific optimum reaction of the medium for every cultivated plant. Only a few investigators have attacked the problem from the opposite side, studying the effect of the plant on the reaction of the growth medium. This side of the problem, however, is of the highest importance, as it may throw light on the causes of soil acidity under natural conditions, a knowledge of which will greatly facilitate the study of the means of controlling the reaction of the soil under cultivated plants. The work here reported was designed primarily to study the effect of plant growth on the medium.

## PREVIOUS WORK

Only a few investigators whose work has a direct bearing on the subject matter of this article will be quoted here.

In 1904 Veitch (11)<sup>2</sup> reported the effect of several kinds of plants on the reaction of the soil. In six years oats followed by buckwheat decreased the "total apparent acidity," as determined by the Veitch method (10), of nearly all the soils, some of the soils originally acid giving an alkaline reaction. On the other hand, beans followed by

<sup>1</sup>Accepted for publication Nov. 1, 1923. Presented in abstract at the meeting of the American Chemical Society in New Haven, April 6, 1923.

<sup>2</sup>Reference by italic numbers is to the literature cited on page 217.

buckwheat during the same period increased the "total apparent acidity" of the soils used.

Breazeale and Le Clerc (3) found that growing wheat seedlings made a sodium nitrate solution alkaline, while solutions of potassium chlorid and potassium sulphate became acid under the same conditions.

Of the investigators using the more recent methods for determining active acidity and alkalinity, Jones and Shive (7) studied the effect of wheat seedlings on the hydrogen-ion concentration of a number of nutrient solutions. Wheat seedlings, after having been grown for five weeks in "Shive's Best" solution, were transferred to the nutrient solutions. The initial hydrogen-ion values of these solutions were recorded, as well as the changes resulting after the seedlings had been in contact with the nutrient for 24 and 52 hours. Invariably the changes were in the direction of decreased acidity. The initial reactions of most of the solutions ranged from  $P_H$  4 to  $P_H$  4.8. At the end of the experiment the reactions ranged from 5.3 to 6.1. Two of the solutions the initial reaction of which had been almost neutral remained practically unchanged under the same conditions.

Hoagland (6), working with barley and several varieties of beans, found that "in every instance, without an exception, nutrient solutions with an initial acid reaction reached approximately neutral reactions, varying from 6.1 to 7.2, after contact with the plant roots for varying periods of time."

Conner and Sears (4), while studying the effect of aluminum salts on plant growth, also found that nutrient solutions with an initial acid reaction tend to become less acid in contact with growing rye, barley, and popcorn seedlings.

Arrhenius (1, p. 81) reported that plant roots change the reaction of the medium, but that the direction of the change depends on the plant used. Rye brought nutrient solutions of original  $P_H$  values 3 and 9 to  $P_H$  5.8; peas brought nutrient solutions of original  $P_H$  values 3 and 8 to 4.5; and corn brought nutrient solutions of original  $P_H$  values 3 and 7.5 to 6.5.

Later the same author (2) studied rice in like manner. Acid reactions in the nutrient solution used were obtained by adding hydrochloric acid and alkaline reactions by adding sodium hydroxide. In all cases the growth of the rice plant brought the reaction to  $P_H$  6.2.

On the other hand, Olsen (8) reports that the kind of plant used does not affect the direction in which change of reaction takes place. He concluded that the direction depends primarily on the source of nitrogen in the nutrient solutions. When ammonium chlorid or nitrate is used, all 12 plant species tried caused the solutions to become more acid, although some plants produced the change more rapidly than others. When sodium nitrate was used, however, the solutions uniformly became more alkaline.

Results obtained in the Bureau of Chemistry (Table I) show that factors other than those given by Arrhenius and Olsen (the kind of plants and source of nitrogen) affect the direction of the change in reactions produced in nutrient solutions under the influence of growing seedlings.



TABLE I.—Changes produced by wheat seedlings in hydrogen-ion concentration of a nutrient solution as affected by its concentration and its initial reaction<sup>a</sup>

Concentration of solution.	Reaction.		
	Initial.	After 1 day.	After 3 days.
	P <sub>H</sub> .	P <sub>H</sub> .	P <sub>H</sub> .
Undiluted.....	5.5	5.4	5.2
Do.....	5.1	4.8	4.6
Diluted 5 times.....	5.5	5.2	4.1
Do.....	5.1	3.9	3.6

<sup>a</sup> The acidity data are reported in this and the other tables in the P<sub>H</sub> system, not because this system is believed to be the best for the purpose, but because other workers are using it and its use facilitates comparison of these results with theirs.

The nutrient solution used had the following composition:

	Gm.
Calcium nitrate.....	2.7
Di-potassium phosphate.....	1.5
Magnesium sulphate.....	.6
Potassium chlorid.....	.75
Ferrous sulphate.....	.01

Twenty wheat seedlings were used for each culture. They were about 4 days old when transferred from tap water to the solution. The nutrient solution was used in two concentrations—full strength and diluted five times. The initial reaction of the full-strength solution, expressed in the P<sub>H</sub> system, was 5.5. The other initial reactions were produced by the addition of hydrochloric acid. Variations in the changes of reaction were obtained while using the same plant and solutions of the same composition. The changes were clearly affected by the initial concentration of the nutrient solution as well as by its initial reactions. The general tendency toward increasing acidity, it appears, was due to the use of very young seedlings, most of the investigators cited having worked with seedlings of more advanced age than those used here. In its present phase, however, this investigation deals primarily with the causes of which the changes under discussion are the result.

Very little work has been done to determine the causes of the changes in reaction which occur in nutrient solutions under the influence of growing seedlings. Arrhenius (1, 2) assumed that these changes are due to root excretions which are regulatory and adapt the reaction of the medium to the needs of the plant. He did not, however, bring any evidence to support this assumption. His finding that the reaction of the soil in immediate contact with roots is different from its average reaction in the same vicinity does not prove that the change is due to neutralization. Selective absorption, which is the alternative, would explain the observations just as well.

Breazeale and Le Clerc (3), and Hoagland (6) also, concluded on the basis of chemical analyses that the changes of reaction produced by growing seedlings in the medium of growth are due to selective absorption. There is, however, some question as to the reliability of direct chemical analysis in dealing with the minute quantities involved, for instance, in a change from P<sub>H</sub> 5.6 to P<sub>H</sub> 6.8, as was the case in Hoagland's experiment.

## EXPERIMENTAL WORK

## PROCEDURE

An indirect method was used in this investigation. Only two explanations of the changes which take place in media under the influence of growing seedlings seem possible—neutralization by root excreta and selective absorption.

In the case of neutralization, the effect produced by the growing seedlings should be independent of the kind of acid used, as long as the initial hydrogen-ion concentration and the dissociation constants are the same. In the case of selective absorption, however, the effect of the seedlings in decreasing acidity should depend upon the chemical composition of the acid used. For instance, it would seem that, other factors being equal, the rate and absolute quantity of diminution in acidity will be greater in solutions of nitric acid than in solutions of hydrochloric acid, since nitrogen is a more essential element of plant food than chlorine. Accordingly, seedlings were grown in solutions of several acids of definite initial hydrogen-ion concentrations, and the changes in active acidity of these solutions were compared at intervals. It is assumed that the principles which govern hydrogen-ion concentration phenomena are essentially the same in solutions of pure acids and alkalies as in more complex nutrient solutions with an acid or alkaline reaction.

Only distilled water solutions were employed, as the use of nutrient solutions with the acids would have led to complications likely to interfere with a clear interpretation of the results. For instance, Conner and Sears (4) added hydrochloric, nitric, sulphuric, and phosphoric acids, respectively, to a Tottingham solution. As this solution contains phosphates, the addition of a strong acid would naturally cause liberation of phosphoric acid to an extent corresponding to equilibrium relations. Consequently these authors investigated largely phosphoric acid.

Another source of complication resulting from the use of a nutrient solution in this connection would be the change in its composition caused by the feeding activities of the seedlings, with the resulting establishment of new equilibria between the elements. Using the present method, practically only two factors need to be reckoned with—the seedlings and the kind of acids.

## PREPARATION OF WATER CULTURES

Wheat seeds were germinated on large perforated aluminum disks floating in tap water, and the seedlings were grown there until they were about 2 inches high. Glasses of approximately 225 cc. capacity containing tap water were covered with paraffined paper perforated with holes smaller than the size of average wheat seeds. The rootlets of the seedlings were then introduced through these holes into the glasses, so that the seeds and plumules rested on the paraffined paper without coming in contact with the liquid media. The seedlings were grown in tap water for about two days to allow good root development and then for a day in distilled water before they were finally transferred to the experimental solutions.

When plants grow in the soil, the seeds are in contact with the same medium as the seedlings, and they may participate in the changes in reaction produced in the medium. In fact, Rudolfs (9) found that

ungerminated seeds changed the initial reaction of single salt solutions. As it was the intention to limit this study to the changes produced by the metabolic processes of growing seedlings, however, this procedure, by which the prevention of contact between seeds and the experimental solutions was assured, seemed best. This procedure also made it possible to grow more seedlings in a single culture than the cork method generally used in studies of this nature.

#### CHANGES OF REACTION IN SOLUTIONS OF VARIOUS ACIDS

The salts of the inorganic acids selected for this experiment are commonly found in soils and are used as fertilizers. The elements which enter into their composition have various functions in plant life and are present in different quantities in plant substance. Their quantitative order is: Nitrogen, phosphorus, sulphur, and chlorin. Functionally, chlorin is considered the least important. A certain number of organic acids were used for the sake of comparison. The experiment was run in duplicate. Samples for analysis were pipetted out through a hole made in the paraffined paper cover. The  $P_H$  values were determined colorimetrically. The checks usually were very good. The figures in the tables represent in the majority of cases averages of two determinations. Five seedlings per culture were used in this experiment. The initial reactions of the acid solutions were  $P_H$  4 and  $P_H$  3.

Of the inorganic acids with an initial  $P_H$  value of 4, nitric acid decreased most in acidity (Table II). The differences involved were small but consistent, and reappeared when the solutions were renewed. The value of  $P_H$  5, obtained twice in the nitric acid solution, indicates that practically all the acidity attained by the addition of the acid was eliminated, as the untreated distilled water used had an acidity of about  $P_H$  5.3, owing to the presence of carbon dioxide. The results with the inorganic acids of the initial  $P_H$  3 series are inconclusive, owing to the small size of the changes produced, which makes the observations more subject to experimental error.

TABLE II.—Changes of reaction produced by growing wheat seedlings in solutions of acids

Acid.	Reaction of solution having an initial $P_H$ of 4.				Reaction of solution having an initial $P_H$ of 3 after 9 days.
	After 1 day.	After 2 days.	After 3 days.	After 10 days.	
	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .
Hydrochloric.....	4.50	4.70	a 4.65	b 4.50	3.0
Nitric.....	4.70	5.00	a 4.90	b 5.00	3.1
Sulphuric.....	4.65	4.70	a 4.70	b 4.75	3.1
Phosphoric.....	4.60	4.80	a 4.80	b 4.70	3.1
Formic.....	4.65	a 6.10	a, c 6.10	6.40	6.4
Acetic.....	4.20	4.50	5.55	6.50	3.1
Oxalic.....	4.80	a 6.10	a, c 6.10	b 6.40	6.4
Succinic.....	4.00	4.40	4.90	6.40	3.0
Benzoic.....	4.00	4.20	4.95	6.45	3.0
Phthalic.....	4.00	4.00	4.60	4.50	3.0

<sup>a</sup> Solution renewed after this reading was obtained.

<sup>b</sup> Seven-day contact.

<sup>c</sup> One-day contact.

The changes which occurred in the organic-acid solutions may have been due partly or wholly to microbiological activity, as it was impracticable to keep the solutions sterile. However, oxalic and formic acids, which have the highest dissociation constants, were acted upon to a greater extent than the other organic acids, especially those in the  $P_H$  3 series.

#### EFFECT OF NUMBER OF SEEDLINGS ON CHANGE OF REACTION

In order to bring out more clearly the differences in the behavior of the individual acids, the foregoing experiment was repeated, using a larger number of seedlings. It was run in duplicate and in two series, one with 10 and the other with 20 seedlings per culture, and was confined to the inorganic acids.

The relative behavior of these acids (Table III) was the same as in the previous experiment. In a general way the rate of response was proportional to the number of seedlings with both of the initial reactions.

In the 20-seedling series, with an initial value of  $P_H$  4, the difference in behavior between nitric acid and the other acids appeared only after the solutions had been renewed twice. At this period, however, the difference between the decreased acidity of the nitric-acid solution and that of the other acids was much more pronounced than in the previous experiment.

In the solutions having an initial  $P_H$  3 value, nitric acid was again more affected by the action of the seedlings than the other acids. Numerically the changes in total values and the differences are small, but quantitatively they are larger than those which took place in the solutions with the initial  $P_H$  4 value. Taking the hydrogen-ion concentration of pure water as a unit, as suggested by Wherry and Adams (12, 13), the decrease in acidity, for example, from  $P_H$  3 to 3.1 would constitute a loss of 2,000 units per liter, while the entire decrease in acidity from 4 to 7 would be only 1,000 units. The fact that losses in acidity designated by the same  $P_H$  numerals vary in actual magnitude, depending upon the  $P_H$  range in which they occurred, is not always realized. Thus, Conner and Sears (4) believed that greater decreases in acidity were produced by growing seedlings in solutions of phosphoric acid with an initial hydrogen-ion concentration of  $P_H$  3.9 and 4.2, which were reduced to 6.3 and 6.4, than in solutions of the same acid with an initial hydrogen-ion concentration of  $P_H$  3.2 and 3.6, which came down to 3.5 and 4.1. Actually, however, the case is just the reverse. Figured on the same basis, the first two transformations involve losses of 1,245 and 625 units, while the last transformations involve losses of 3,150 and 1,700 units.

Nevertheless, it is significant that, while in the solutions of the lower hydrogen-ion concentrations in these experiments, as well as in those of Conner and Sears (4), the acidity originally present was practically exhausted, in the case of the higher concentrations the action of the seedlings seemed to have stopped while appreciable amounts of acid were still left in the solutions. But the seedlings also lost their power to reduce acidity in the solutions of the lower initial hydrogen-ion concentrations of these experiments when they were renewed several times. Evidently there are certain limits to the absolute quantities of acid upon which seedlings can act. Within these limits, however, nitric acid was more subject than the other acids to that action of the seedlings which is responsible for decreasing acidity.

TABLE III.—Effect of number of wheat seedlings on change of reaction produced in inorganic acid solutions

Acid.	Initial P <sub>H</sub> .	Reaction with 20 seedlings.						Reaction with 10 seedlings.					
		After 1 day.	After 2 days.	After 4 days (1-day contact). <sup>a</sup>	After 5 days (1-day contact).	After 6 days (2-day contact).	After 10 days (1-day contact).	After 1 day.	After 2 days.	After 4 days.	After 5 days (1-day contact).	After 6 days (2-day contact).	After 10 days (6-day contact).
Hydrochloric.....	4.0	4.9	b 5.05	b 4.7	4.0	b 4.0	4.4	4.7	4.8	b 4.9	4.0	4.0	4.3
Nitric.....	4.0	4.9	b 5.15	b 5.15	4.9	b 5.0	4.4	4.7	4.0	b 5.2	4.4	4.4	4.25
Sulphuric.....	4.0	4.9	b 5.0	b 4.7	4.2	b 4.3	4.4	4.7	4.8	b 4.9	4.2	4.2	4.3
Phosphoric.....	4.0	5.0	b 5.1	b 4.85	4.0	b 4.3	4.0	4.7	5.0	b 5.0	4.0	4.1	4.0
Hydrochloric.....	3.0	3.0	3.0	3.0	3.1	3.1	3.1	.....	.....	.....	.....	.....	3.0
Nitric.....	3.0	3.0	3.0	3.1	3.2	3.2	3.3	.....	.....	.....	.....	.....	3.2
Sulphuric.....	3.0	3.0	3.0	3.1	3.2	3.2	3.2	.....	.....	.....	.....	.....	3.0
Phosphoric.....	3.0	3.1	3.1	3.1	3.2	3.2	3.2	.....	.....	.....	.....	.....	3.0

<sup>a</sup> Specified days of contact refer to P<sub>H</sub> 4 series only.<sup>b</sup> Solution renewed after this reading was obtained.

## EFFECT OF PREVIOUS TREATMENT OF SEEDLINGS ON CHANGES IN REACTION OF ACID SOLUTION

The behavior of seedlings which had been grown previously in a complete nutrient solution, as compared with that of seedlings which had previously been grown in incomplete nutrient solutions, defective in chlorin, nitrogen, sulphur, and phosphorus, was studied. For instance, the seedlings which were to be grown in a solution of hydrochloric acid were first grown in a nutrient solution which was lacking in chlorin.

The nutrient solution used was diluted five times. The defective solutions were made up so that they differed as little as possible from the complete solution, except for the missing element. The seedlings were divided into two series: One series was grown for five days in the complete nutrient solution, and the other in the defective solutions. All seedlings were then grown for a day in distilled water and finally transferred to the acid solutions with an initial reaction of  $P_H$  4. After the changes for two successive days had been recorded the acid solutions were renewed and the changes in  $P_H$  values were again recorded after a one-day and after a three-day contact. All seedlings were then transferred to the complete nutrient solutions diluted 10 times. Here they grew for two days, after which they were transferred to the complete and defective nutrient solutions, diluted 10 times. Two days later they were transferred to the acid solution with the initial reaction of  $P_H$  3.6, in which they were kept for three days, when the changes in reaction were again recorded.

TABLE IV.—*Effect of complete and incomplete nutrient solutions in which seedlings had been previously grown on the changes produced in inorganic acid solutions<sup>a</sup>*

Acid.	Previous condition of solution.	Reaction.				
		After 1 day.	After 2 days. <sup>b</sup>	After 3 days <sup>c</sup> (1-day contact).	After 5 days (3-day contact).	After 12 days (3-day contact).
		$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .
Hydrochloric.....	{Complete.....	4.65	4.90	4.45	4.85	4.25
	{Complete—Cl.....	4.60	4.70	4.50	4.65	3.95
Nitric.....	{Complete.....	4.60	5.10	4.50	4.85	3.95
	{Complete—N.....	4.55	4.70	4.60	4.70	3.85
Sulphuric.....	{Complete.....	4.65	5.40	4.45	4.85	4.00
	{Complete—S.....	4.65	4.95	4.50	4.65	3.90
Phosphoric.....	{Complete.....	4.70	5.50	4.60	5.15	5.20
	{Complete—P.....	4.60	4.80	4.65	4.70	4.00

<sup>a</sup> Initial  $P_H$  4.0; number of seedlings, 15.

<sup>b</sup> Solutions renewed after these readings were obtained.

<sup>c</sup> All seedlings were held in complete (1:10) solution for 2 days, then for 2 days in complete and defective (1:10), and were then transferred back to acid solutions with an initial reaction of  $P_H$  3.6.

The results (Table IV) show that the previous growth of the seedlings in a complete nutrient solution did not affect their activity, so far as their ability to lower the initial hydrogen-ion concentration was concerned. The previous growth of the seedlings in defective solutions, for the purpose of creating an avidity for the elements of the acids with which they were subsequently to be brought in contact, resulted, contrary to expectation, in a depressed activity with reference to changes

in reaction of the acid solutions. This is shown by the three final readings (before solutions were renewed or discarded) which give consistently higher acidities for the seedlings grown previously in defective nutrient solutions. The defectiveness of the previous nutrient solution evidently created in the seedlings some functional disturbance either general or with reference to their behavior toward the elements lacking.

The most significant result obtained in this experiment, however, is that in the case of the older seedlings nitric acid had ceased to be affected by the action of the seedlings to a greater extent than the other acids. Its place was taken by phosphoric acid, as was shown by the three final readings in the series which had been grown in the complete nutrient solution.

The initial acidity of phosphoric acid solution was reduced from  $P_H$  3.6 to  $P_H$  5.2 in the final reading, a reduction which is strikingly greater than any obtained in the case of the other acid solutions. The differences in the behavior of the various acids were thus brought out more distinctly. The preference of the seedling for the different acids was also shown to vary with the conditions and with the stage of growth.

#### EFFECT OF AGE OF SEEDLINGS ON CHANGES IN REACTION IN ACID SOLUTIONS

The effect of the age of seedlings on their behavior toward nitric and phosphoric acids, which was unexpectedly brought out by the previous results, was studied further.

The usual procedure was followed. After the seedlings had been transferred to glasses and grown in tap water for three days, the roots were kept in contact with distilled water for two hours. The seedlings were then divided into two series. The seedlings of the first series were transferred to the acid solutions immediately. The seedlings of the second series were transferred to the acid solutions when 15 days old, after having been grown for 10 days in the nutrient solution diluted five times. The initial reaction of the acid solutions used was  $P_H$  3.6, which was more suitable than the initial reaction of  $P_H$  4, as the initial acidity was thereby increased 250 per cent and the changes produced were more pronounced.

TABLE V.—Effect of age of seedlings on changes produced in inorganic acid solutions<sup>a</sup>

Acid.	Initial age of seedlings.	Reaction.							
		After 1 day.	After 2 days. <sup>b</sup>	After 4 days (2-day contact).	After 6 days (4-day contact).	After 11 days (7-day contact). <sup>b</sup>	After 13 days (2-day contact). <sup>c</sup>	After 17 days (2-day contact).	After 20 days (5-day contact).
	Days.	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .	$P_H$ .
Hydrochloric.....	5	4.05	4.00	3.6	3.70	.....	.....	.....	.....
Nitric <sup>d</sup> .....	5	4.40	5.10	4.3	4.70	.....	.....	.....	.....
Sulphuric.....	5	3.90	4.10	3.6	3.65	.....	.....	.....	.....
Phosphoric.....	5	4.15	4.45	3.9	3.95	.....	.....	.....	.....
Hydrochloric.....	15	.....	.....	.....	.....	4.80	3.90	4.00	4.35
Nitric.....	15	.....	.....	.....	.....	4.75	4.00	4.20	4.60
Sulphuric.....	15	.....	.....	.....	.....	4.90	3.90	4.30	4.50
Phosphoric.....	15	.....	.....	.....	.....	5.20	4.20	4.45	6.15

<sup>a</sup> Initial  $P_H$ , 3.6; number of seedlings, 20.

<sup>b</sup> Solution renewed after these readings were obtained.

<sup>c</sup> Transferred to complete nutrient solution (1:5) for 2 days and then back to acid solutions.

<sup>d</sup> Initial  $P_H$ , 3.5.

The results (Table V) show again and more clearly that the preference of the seedlings at an early stage of growth is for nitric acid and at a later stage for phosphoric acid. The age factor in this experiment also includes the factor of previous mineral nutrition. As a rule, however, the two are inseparable, as age in the sense of advanced stage of growth can not be measured merely by the number of days which elapse from the time of germination.

#### DISCUSSION OF EXPERIMENTAL RESULTS

The fact that the activity of seedlings in decreasing the initial acidity of the medium is preferential, resulting in the greatest decrease in acidity in one acid at one set of conditions and in another acid at another set of conditions, indicates that the cause of these changes is preferential absorption by the plants.

The term "preferential absorption" seems more appropriate here than the commonly used term "selective absorption." "Selective absorption" might imply that some substances are excluded, while "preferential absorption" indicates merely a higher rate of absorption of one substance as compared with that of another.

If the changes in the initial reaction of the acid solutions were a result of neutralization by secreta from the roots, they would be expected to be controlled by the active acidity factor only, and different acids with the same initial hydrogen-ion concentration and the same dissociation constants would behave alike. If the preferential action of the plants were in favor of nitric acid throughout, reduction by microorganisms might have suggested itself. It has been shown elsewhere (4), however, that the possibility of nitrate reduction is eliminated under the conditions of these experiments. But the fact that the preference of the older seedlings is for phosphoric acid lends additional strength to the absorption theory, which is further supported by the fact that in plant life nitrogen and phosphorus are, at least quantitatively, the most essential of the acid-forming elements dealt with in this investigation.

#### SUMMARY

Wheat seedlings were grown in solutions of hydrochloric, nitric, sulphuric, phosphoric, formic, acetic, oxalic, succinic, benzoic, and phthalic acids. The changes in reaction produced by their growth, recorded at certain intervals, show that:

Of the inorganic acids, the greatest changes were produced in nitric acid at early stages of growth of the seedlings and in phosphoric acid at later stages. Phosphorus and nitrogen being the most essential elements of plant growth contained in the acids used, it may be concluded that the changes in initial reaction produced by plants in the medium in which they grow are due to absorption rather than to neutralization.

The previous growth of the experimental seedlings in nutrient solutions deficient in acid-forming elements diminished their ability to decrease the acidity of the acid solutions. Apparently the deficiency of the previous nutrient solutions produced functional disturbances in the seedlings.

The greatest changes from the initial reactions were produced in the solutions of the organic acids. This, however, may have been due partly or wholly to microbiological activity and needs to be studied further.



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# THE NUTRITIVE PROPERTIES OF WILD RICE (*ZIZANIA AQUATICA*)<sup>1</sup>

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## INTRODUCTION

Wild rice, a cereal which is distantly related to the cultivated rice, is an annual grass growing in shallow lakes and sluggish streams over a widely extended territory in North America. It is used rather extensively as a food by some of the Indian tribes of the upper Mississippi Valley and, to a more limited extent, in hotels and in private homes during the game seasons. It also serves as an important source of food for wild water fowl.

Eight years ago the Division of Agricultural Biochemistry undertook an investigation of wild rice, both because the cereal grows quite extensively in Minnesota and because it was desirable to ascertain its food value in order to determine whether it was advisable to improve the methods of its cultivation, harvesting, curing, and marketing, so that it could become of agricultural importance. At that time, as at the present, practically no means were employed to preserve the natural rice beds or to plant the rice in new localities, and the methods of harvesting and curing were very crude and wasteful.

Wild rice usually matures in the latter part of August or in September after the first frost. Shortly before this time the Indian harvesters go into the rice beds and tie the standing stalks into small bunches; then, when the grain is sufficiently matured, they return in their canoes and, holding the bunches over their boats, knock the ripened kernels into the bottoms of the boats. Only about 50 per cent of the ripe grains fall into the boats, the rest being lost in the mud of the lake.

Since the rice is gathered before it is fully ripened, it must be cured or artificially ripened. This process also aids in removing the tenacious hull. The grain is cured, (1) by the sun, (2) by smoke and heat from a slow fire under the rice, which is spread on a framework above, and (3) by parching or popping in a metal vessel. After drying, the hulls are threshed off either by treading or by striking with paddles. The hulls are finally blown off by the wind or with a fan. Such is the primitive method used by both the Indian and the white man to gather and cure the rice.<sup>2</sup>

## CHEMICAL ANALYSIS OF WILD RICE

When this investigation was begun, in 1915, the biological method for the analysis of a foodstuff was unknown. Later, when this method of appraising the value of foods had developed, the investigation was

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<sup>2</sup> A general description of wild rice, its habitat, production, etc., is given in JENES, A. E., *THE WILD RICE GATHERERS OF THE UPPER LAKES*. In 19th Ann. Rpt. Bur. Amer. Ethnol. (1897-98), pt. 2, p. 1013-1137, illus., 1900. A brief account of the plant, its culture, etc., is found in CHAMBLISS, C. E., *WILD RICE*. U. S. Dept. Agr. Circ. 229, 16 p., illus., 1922.

expanded to include a biological analysis of the wild rice. The first part of the investigation was therefore confined to the chemical composition of the cereal. Four samples of rice were secured for analysis, two being obtained in the local markets and two direct from the Indians in the region of the Minnesota rice lakes. Three of these samples were of quite uniform appearance, consisting of slender cylindrical kernels varying from one-half inch to nearly one inch in length, and were of a dark slate color. The remaining sample (No. 2) was quite different in its physical appearance, the kernels being very long and thick, and many of the grains being parched to such a degree that they resembled puffed cereals.

Table I shows the results of the analysis of the four samples of wild rice, and, for comparison, an analysis of a polished and an unpolished cultivated rice. It is seen that the proportions of protein and soluble carbohydrates in the wild rice are considerably greater than in either sample of the cultivated variety. The soluble carbohydrates of the rice are probably formed in large measure during the parching process which is commonly used in removing the hulls. The ether extract of the wild variety is approximately double that of the polished cultivated rice, but considerably less than that of the unpolished cultivated variety. Much of the embryo is doubtless removed in the wild rice by the parching process to which kernels are subjected in order to remove the hulls, but, since the parching process is not at all uniform, the embryo is not entirely removed, as it is in the polishing of the cultivated rice.

The amount of inorganic material is considerably higher in the case of the wild rice than in that of the cultivated rice. Table II shows the distribution of the inorganic elements in the ash of the two varieties. For comparison, an analysis of dried skim milk is also included in this table.

TABLE I.—*Comparison of chemical composition of wild rice and cultivated rice*

Sample No.	Moisture.	Ash.	Protein.	Ether extract.	Fiber.	Starch.	Soluble carbohydrates as dextrose.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
1.....	7.74	1.09	13.36	0.455	1.39	65.26	2.98
2.....	7.85	1.38	13.97	.893	1.41	61.69	3.69
3.....	8.93	1.17	14.62	.718	1.94	60.47	2.33
4.....	7.83	1.25	14.40	.658	1.29	62.03	2.93
Cultivated unpolished rice.....	12.22	1.01	5.04	2.01	1.08	69.50	.85
Cultivated polished rice.....	12.30	.40	8.0	.30	.20	79.00	.....

TABLE II.—*Distribution of inorganic elements in wild and cultivated rice in comparison with dry skim milk*

Substances compared.	Ca.	Mg.	K.	Na.	P.	S.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Wild rice.....	0.018	0.080	0.055	0.064	0.424	0.252
Cultivated polished rice.....	0.008	0.027	0.069	0.021	0.102	0.105
Dry skim milk.....	1.33	0.147	1.27	0.488	0.979	0.357

As the composition of the inorganic matter of milk is adequate to meet the needs of the growing animal, it is readily seen that both varieties of rice have very inadequate amounts of the essential ash constituents. This point will be discussed further in connection with the biological analysis of the rice.

#### BIOLOGICAL ANALYSIS OF WILD RICE

Since it is now recognized that a chemical analysis of a foodstuff is very inadequate for judging its food value, the investigation of the wild rice also included a biological analysis. From this analysis it is possible to compare the value of this cereal with that of many of the cereals in common use, and thus evaluate it as a food product.

The method of procedure was that commonly used when it is desired to determine the deficiencies of a single foodstuff. Rice was fed, with no additions; and when it became evident that growth could not be induced on such a food, additions of known purified food constituents were made singly and in groups until a food was produced which gave satisfactory growth. The results of this procedure make it clear that the dietary properties of wild rice are much like those of our common cereals, but somewhat better than those of polished rice.

The wild rice used in the feeding experiments was obtained in the local market, ground to a fine meal, and fed, both unmixed and with certain additions, to young rats weighing from 70 to 80 gm. The rats were kept on sawdust bedding in clean, well-ventilated cages. The efficiency of these different rice rations in promoting growth in the rat is set forth in figures 1 and 2.

The ration of the rats of Lot 1 (fig. 1) consisted of wild rice with no additions. This proved to be an entirely inadequate food, for the greater number of animals in this lot constantly lost weight, and by the end of the third week were in an extremely emaciated condition.

The first addition that it seemed advisable to make was that of inorganic constituents, since the chemical analysis of the rice had shown it to be very deficient in the mineral elements essential for growth. As the ash analysis of the wild rice (Table II) did not differ greatly from that of polished rice, the same salt mixture that McCollum and Davis<sup>3</sup> used with polished rice was added to the cereal. This salt mixture consisted of sodium chlorid 5 parts, dipotassium phosphate 12.1 parts, monocalcium phosphate 2.56 parts, calcium lactate 29.44 parts, and ferric citrate 1 part.

The ration of the rats of Lot 2 (fig. 1) contained 4 parts of this salt mixture and 96 parts of wild rice. Although the addition to the inorganic content of the ration greatly improved the growth of the rats, this growth was still far from normal. It is interesting to compare these growth curves with those obtained by McCollum and Davis<sup>4</sup> on a ration consisting of polished rice with the addition of the same salt mixture in the same proportion. Their rats not only failed to respond to this addition but steadily lost weight, thus indicating that the cultivated polished rice is more deficient in necessary food constituents than the wild variety.

<sup>3</sup> McCOLLUM, E. V., and DAVIS, M. THE NATURE OF THE DIETARY DEFICIENCIES OF RICE. *IN* JOUR. Biol. Chem., v. 23, p. 195. 1915.

<sup>4</sup> McCOLLUM, E. V., and DAVIS, M., *OP. CIT.*, p. 195.

As it was evident from the growth curves of this lot that the rice-salt diet was inadequate to promote normal growth, vitamin A was added, for the reason that cereals as a class are deficient in this factor. A ration consisting of wild rice 91 parts, salt mixture 4 parts, and butter fat 5 parts was fed to the rats in Lot 3 (fig. 1). The addition of the butter fat made a great improvement in the rice-salt ration, as almost normal growth was obtained for a period of two months and pregnancy was secured twice in the case of one individual. However, the rats of the first litter were all born dead, and in the second pregnancy the rat died before the young were born, autopsy showing nine fetuses at term.

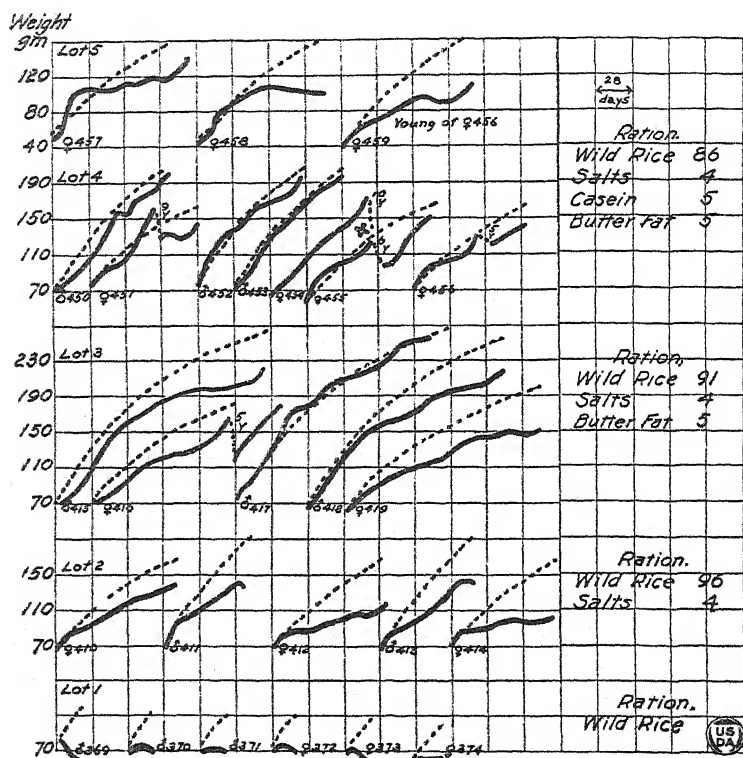


FIG. 1.—Rate of growth of rats on rations consisting of wild rice alone and wild rice with various additions. + indicates death of rat 454, Lot 4; y=young.

This result, together with the fact that none of the growth curves in this group approached the normal after the second month, showed that the ration was still deficient. Nevertheless, comparison of these growth curves with those of McCollum and Davis,<sup>5</sup> rats on a similar ration containing polished rice shows that the wild rice with the addition of proper quantities of salts and butter fat is a much better ration than polished rice with the same additions. The growth curves of Lot 3 indicate that the proteins and the quantity of vitamin B are fairly adequate, because if either one or the other or both were deficient, such good growth could not have been attained.

<sup>5</sup> McCOLLUM, E. V., and DAVIS, M., *OP. CIT.*, p. 199.

Considering that many of the cereals not only are low in inorganic constituents and vitamin A but also have proteins of poor quality, casein was the next addition made to the rice-salt-vitamin A ration. The new ration, consisting of wild rice 86 parts, salt mixture 4 parts, butter fat 5 parts, and casein 5 parts, was fed to the rats of Lot 4 (fig. 1). The addition of the casein allowed the rats to grow normally, although reproduction and rearing of young were not satisfactory. Each of the females had one litter, but out of a total of 29 rats in the second generation only 3 could be reared to adult size, the other 26 either being born dead, dying shortly after birth, or being destroyed by the mother.

The three rats of the second generation, Lot 5 (fig. 1), had a total weight of 147 gm. at the end of the fifth week, when they were weaned and given the mother's ration. For the first four weeks their growth was normal, but after this period growth was much below normal. This failure to grow normally was probably due to the fact that the rats had a lung infection which was manifesting itself in our rat colony at the time, although it might have been due to a dietary deficiency which prevented normal development in the second generation.

The excellent results obtained by the addition of the small amount of casein showed that the protein of the wild rice was not wholly adequate for growth, and in fact was probably the only other factor deficient in the cereal. The two additions already made, inorganic salts and vitamin A, with the further addition of protein, make wild rice a food better suited to promote growth than the same additions to polished cultivated rice. Thus, McCollum and Davis<sup>6</sup> found that the addition of 2 per cent of casein to a ration consisting of polished rice, salt mixture, and butter fat did not lead to growth, but that growth was greatly stimulated by the addition of wheat embryo. The growth curves of their rats,<sup>7</sup> whose

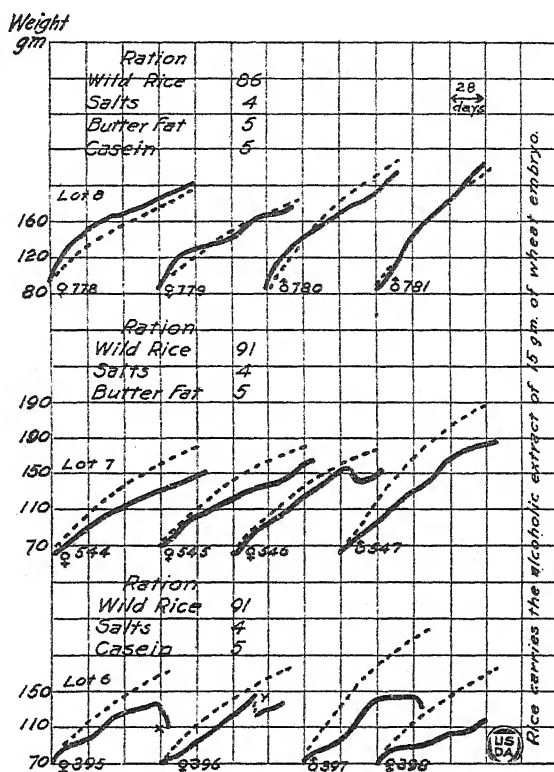


FIG. 2.—Growth curves of rats, illustrating the effect of additions of vitamin B to wild rice rations which had been modified by casein, butter fat, and mineral salts. + indicates death of rat 395, Lot 6; y denotes young.

<sup>6</sup> McCOLLUM, E. V., and DAVIS, M., *OP. CIT.*, p. 200.

<sup>7</sup> McCOLLUM, E. V., and DAVIS, M., *OP. CIT.*, p. 191, 223.

ration consisted of polished rice 64 parts, casein 13.4 parts, salt mixture 2.4 parts, butter fat 5 parts, agar 2 parts, and dextrin 13.2 parts, carrying the extract of 5 gm. of wheat embryo per 100 gm. of ration, were no better than the growth curves of the rats of Lot 4 of this experiment, whose ration contained but 5 per cent of additional protein and no addition of vitamin B.

Although cereals contain an abundance of vitamin B, it was thought that wild rice might be deficient in this factor because the parching process, often used to remove its hulls, might also char the embryo, which is probably the principal source of this vitamin. A different series was carried out, therefore, along the lines described in connection with Figure 1, using wild rice as before, with butter fat and casein, but with the addition of the alcoholic extract of 15 gm. of wheat embryo to each 100 gm. of ration. The same salt mixture was fed as before. The results of these feedings are graphically represented in figure 2.

The ration of Lot 6 (fig. 2) consisted of wild rice 91 parts, salt mixture 4 parts, and casein 5 parts. The rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. The failure of these rats to grow normally was due to the absence of vitamin A.

The ration of Lot 7 (fig. 2) consisted of rice 91 parts, salt mixture 4 parts, and butter fat 5 parts. As before, the rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. This addition of the vitamin B preparation was made to ascertain whether the ration of Lot 3 (fig. 1) was deficient in this factor. The addition caused no improvement. This result, together with the fact that excellent growth was obtained when casein was added to the ration of Lot 3, producing the result shown in Lot 4, proves that wild rice contains an adequate amount of vitamin B for growth.

The ration of Lot 8 (fig. 2) consisted of rice 86 parts, salt mixture 4 parts, butter fat 5 parts, and casein 5 parts. Again the rice carried the alcoholic extract of 15 gm. of wheat embryo per 100 gm. of ration. This ration was fed in order to find out if the ration of Lot 4 (fig. 1) was deficient in vitamin B. As no better growth was obtained, it can be concluded that the ration of Lot 4 is not deficient in this factor. It was unfortunate that the experiment had to be terminated before reproduction occurred.

#### CONCLUSIONS

- (1) Wild rice is not an adequate food.
- (2) Although chemical analysis shows a higher percentage of protein present than in many cereals, wild rice resembles many other cereals in containing proteins of rather low biological value. It further resembles other cereals in containing inorganic material unsuitable for the promotion of growth, and in being very deficient in vitamin A, although enough of this vitamin is present to prevent xerophthalmia.
- (3) Wild rice has a greater food value than the cultivated polished rice, because its proteins are of better quality and because it contains adequate amounts of vitamin B for animal growth, which is not true of the polished cultivated rice.



# A BACTERIAL BLIGHT OF GLADIOLI<sup>1</sup>

By LUCIA McCULLOCH

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## INTRODUCTION

In the summer of 1922 a disease that was causing serious injury to the leaves of gladioli was reported by a large grower of these plants in the Middle West. The disease if present at all in previous years had not attracted attention.

In 1923 in the same fields, and especially in poorly drained areas, the disease appeared on some young stock and a large number of these plants were destroyed. However, the infection abated rather suddenly and caused no further damage. Occasional cases of infection were noted on the older stock.

In early September (1922) when the affected fields were examined by the writer, the leaves had a distinctly burned appearance. Closer observation showed them to be variously spotted, browned and ragged. Many leaves were heavy and stiff with adhering soil particles.

Young stock appears to be much more susceptible to this disease than mature plants. Of 16 varieties studied in the field, the young stock of 15 varieties was severely attacked, while mature plants of the same varieties showed only slight spotting or no infection. One exception was noted, mature plants of the variety Schwaben were nearly as badly infected as the young stock. On the contrary, no trace of this disease was found on either young or mature plants of the variety Mrs. Frank Pendleton, although these were growing in the same fields and under the same general conditions.

Microscopic examination of the discolored areas on the leaves revealed great quantities of bacteria in the tissues.

In a search through the literature of plant diseases, no description has been found that resembles this blight of the gladiolus and at present this mid-west farm is the only locality known to the writer where the disease occurs. But since it is a serious menace to gladiolus culture and may be present elsewhere, it seemed worthy of further investigation. Careful observations were made in the fields and considerable material was collected for study. During the succeeding months experiments on plants in greenhouses and in outdoor beds and studies in the laboratory have established the following facts concerning the disease.

Lesions are first visible as narrow, horizontal, water-soaked spots (Pl. 1, A); the later progress of the infection is mostly in a longitudinal direction so that the spots become more or less regularly formed squares or rectangles (Pl. 1, C and E). Adjoining lesions unite until often the entire leaf from the tip to the base is involved. This widespread infection of the leaf is most noticeable in the young stock. All parts of the leaves of blooming stock are also subject to attack, but the infection is quite

<sup>1</sup> Accepted for publication Dec. 14, 1923.

often found only on the middle area of the leaf. As seen in fresh material, these spots are translucent and watersoaked. In reflected light the color is a very dark green; in transmitted light it is a bright green. The translucency of the spots is permanent, but when old and dry the colors are various shades of brown.

Considerable quantities of a bacterial exudate flow from the affected tissues. In quiet, dry air this exudate forms slender, twisted white columns 2 to 10 mm. long. Under moist conditions it forms a viscid film over the leaf. When dry it may be a thin, brittle layer or it may occur in the form of numerous small droplets. Wind-blown soil, tiny insects, and various small particles become embedded in this exudate, often in sufficient quantities to cover the leaf surface. The exudate seems to be composed entirely of bacteria; it dissolves readily in water and doubtless is a factor in the dissemination of the disease.

The name *Bacterium gummisudans* proposed for the causal organism of this disease, was suggested by the copious exudate from the lesions.

#### ISOLATION AND REINFECTION

Yellow, viscid bacteria were repeatedly and easily isolated from the lesions and from the exudate, and numerous inoculation experiments have proved these bacteria to be the cause of the disease.

From lesions produced by pure culture inoculations the organism was reisolated on agar-poured plates and with subcultures from single colonies typical reinfections were obtained. Repeated experiments in inoculating, reisolating, and reinfesting leave no doubt either as to the cause of the disease or as to the general character of the lesions.

Young plants grown from cormels were readily infected. Older plants were more resistant. Of six varieties of mature plants used for inoculation experiments, Schwaben was the only one that became generally infected; Mrs. Francis King was very slightly infected. On Mrs. Frank Pendleton no infection occurred. These inoculation results agree with the field observations in regard to relative susceptibility of young and mature plants and of varieties.

Iris, hyacinth, and barley were inoculated but no infections resulted.

#### MORPHOLOGY

The organism is a short, actively motile rod, occurring singly and in pairs in the host and in most culture media. In beef bouillon it forms long chains. It measures when stained with carbol fuchsin 1 to  $2.8\mu$  by  $0.6$  to  $0.8\mu$ ; no spores have been seen. Capsules are formed on beef peptone agar and on potato dextrose agar. The single polar flagellum is 3 to  $9\mu$  long. The flagella were demonstrated by the use of Casares-Gil's staining method. It is Gram-negative and not acid-fast.

#### CULTURAL CHARACTERS

On peptone beef<sup>2</sup> agar the colonies are pale yellow (amber yellow<sup>3</sup>), circular, transparent; the surface is smooth and the interior has concentric striations (Pl. 2, F). With age the growth becomes less transparent and the striae disappear. Buried colonies are oval to spindle-shape and have definite margins.

<sup>2</sup> All the beef media used in this study were made from fresh beef infusion with addition of 1 per cent Difco peptone corrected to +14 to +18 Fuller's scale ( $P_H$  6.8 to 6.4).

<sup>3</sup> RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

Beef bouillon clouds only moderately but forms a thick, yellow rim and a thin pellicle. The rim and pellicle are extremely viscid. The growth is viscid in most media but especially so in beef infusion peptone media.

Liquefaction of gelatin is not rapid at 18 to 20° C. Colonies on plates showed first signs of liquefaction on the fifth day. In stab cultures the upper fifth of the medium was liquefied by the seventh day. Blood serum becomes translucent and partly liquefied.

On potato cylinders the growth is pale yellow and abundant, usually covering the cylinder and filling the water with a thick growth. The potato is slightly browned.

A slight acidity is produced in milk cultures. A soft, smooth curd forms, but it is rapidly and completely digested. As the casein is digested the medium becomes translucent, but the translucency is obscured by the large quantity of almost opaque, viscid substance that practically fills the medium. Heavy, yellow, viscid rims and yellow sediment develop in milk cultures. Tyrosin crystals are formed.

Litmus in milk is slowly reduced.

No gas is formed from dextrose, saccharose, lactose, maltose, galactose, or glycerin. Slight amounts of acid are produced in cultures containing dextrose and saccharose. With lactose and glycerin the reaction is consistently alkaline.

Growth is scanty in Cohn's and Uschinsky's solutions; scanty and fugitive in Fermi's solution.

Ammonia and hydrogen sulphid are produced in cultures. Several tests for indol have given negative results. Controls of *B. coli* gave positive indol reactions.

Nitrates are not reduced.

Sodium chlorid is not well tolerated, 1 per cent definitely reducing growth, and no growth occurring above a 2 per cent concentration.

The optimum reaction for growth in peptone beef bouillon is +14 to +17 ( $P_H$  6.8 to 6.5).<sup>4</sup> Even under only slightly adverse conditions growth is slow, and no growth occurs if the media is much below or above the optimum. For example, in one of the experiments for the determination of the thermal death point, peptone beef bouillons titrating +6, +10, +15, and +17 ( $P_H$  7.6, 7.2, 6.7, and 6.5) were used. All four were made from the same beef infusion stock. The tubes had the same amount of inoculum, and the heating tests were simultaneous. Cultures in +15 and +17 survived higher temperatures than those in +6 and +10. In the controls growth was retarded in the +6 and +10 and never became so heavy as in the +15 and +17 media. The minimum is at 0 ( $P_H$  8.2), the maximum at +25 ( $P_H$  5.7).

The toleration of organic acids was tested by adding 0.1 and 0.2 per cent, respectively, of citric, malic, and tartaric acids to a neutral bouillon base. The bacteria grew normally in the 0.1 per cent acids (+15,  $P_H$  7.0) and not at all in the 0.2 per cent (+27,  $P_H$  5.7).

#### TEMPERATURE RELATIONS

In beef bouillon the optimum temperature is near 30° C.; the minimum is below 2°. Growth begins but does not continue at 36°. The thermal death point is near 50°.

<sup>4</sup>QUIRK, Agnes J., and FAWCETT, Edna H. HYDROGEN-ION CONCENTRATION VS. TITRATABLE ACIDITY IN CULTURE MEDIA. *In Jour. Infect. Diseases*, v. 33, p. 20. 1923.

## DESICCATION

The majority of young cultures dried on cover glasses were dead inside of 24 hours, and all within 3 days.

The bacteria were easily isolated from dry leaves up to 10 days after collection. After 10 days' drying, success in isolation was infrequent. In only 1 case out of 14 was the organism recovered from leaves dried as long as 2 months.

Exposure to sunlight for 15 minutes killed 100 per cent of the organisms.

## RELATION TO HOST TISSUES

Microscopic examination of the lesions shows that the organism attacks the parenchyma. The intercellular spaces and the cavities formed by disintegration of cell walls become packed with the bacteria.

For a study of the early stages, material was secured from plants inoculated by spraying with pure cultures of the bacteria suspended in water. As soon as infection was evident the tissues were collected and fixed. Stained sections of this material show that the bacteria enter by way of the stomata (Pl. 2, B and E). In the earliest stages the bacteria are found only in the stomatal chamber, from which they spread into and fill adjoining intercellular spaces. That some chemical change occurs at this stage in the cell walls adjoining the masses of bacteria is evidenced by their increased affinity for stains. Whether they are later dissolved or only crushed by pressure and so changed chemically as to be vague and doubtful is not yet determined, but cavities occur and become entirely filled with the parasite. In lesions of considerable size no definite cell-wall structure remains in the central space. In some large cavities the bacteria are arranged in layers (Pl. 2, A).

## COMPARISONS WITH SOME SIMILAR BACTERIAL DISEASES

The translucency of the lesions and the yellow color of the bacterial growth suggested that this leaf spot might be identical with some previously described bacterial disease on monocotyledons. However, careful comparisons show that *Bacterium gummisudans* has characters that separate it from the several organisms that it, to a certain degree, resembles. *Bacterium translucens* Jones, Johnson, and Reddy<sup>5</sup> and *Bacterium translucens* var. *undulosum* Smith, Jones and Reddy,<sup>6,8</sup> causing disease on barley and wheat, respectively, are unlike *Bacterium gummisudans* in cultural characters in several media and in the consistency of the bacterial growth. Moreover, *Bacterium gummisudans* does not infect grains. Four varieties of barley were used in the inoculation experiments. Several tests were made, but no infections resulted. Control inoculations on gladioli gave typical infections. *Aplanobacter rathayi* EFS.<sup>7,8</sup> and *Aplanobacter agropyri* O'Gara<sup>9</sup> (on western wheat grass) are nonmotile organisms, while *Bacterium gummisudans* is a motile organism.

<sup>5</sup> JONES, L. R., JOHNSON, A. G., and REDDY, C. S. BACTERIAL-BLIGHT OF BARLEY. *In* Jour. Agr. Research, v. 11, p. 625-643, 2 fig., pl. B (col.), 47-49. 1917. Literature cited, p. 643.

<sup>6</sup> SMITH, ERWIN F., JONES, L. R., and REDDY, C. S. THE BLACK CHAFF OF WHEAT. *In* Science, n. s., v. 50, p. 48. 1919.

<sup>7</sup> SMITH, ERWIN F. BACTERIA IN RELATION TO PLANT DISEASES. v. 3, p. 155-160. Washington, D. C. 1914. (Carnegie Inst. Wash. Pub. 27.)

<sup>8</sup> — AN INTRODUCTION TO BACTERIAL DISEASES OF PLANTS. fig. 12-22. Philadelphia and London. 1920.

<sup>9</sup> O'GARA, P. J. A BACTERIAL DISEASE OF WESTERN WHEAT GRASS, AGROPYRON SMITHII. *In* Phytopathology, v. 6, p. 341-350, pl. 9-13. 1916.

## TECHNICAL DESCRIPTION

*Bacterium gummisudans*, n. sp.

A short rod, rounded at the ends, individual rods 1 to 2.8 $\mu$  by 0.6 to 0.8 $\mu$ ; motile by means of one polar flagellum 3 to 9 $\mu$  long, capsules present, no spores; aerobic. A yellow, viscid growth is formed on culture media; nitrates are not reduced; casein in milk is digested; gelatin is liquefied; acid is formed from dextrose and saccharose; ammonia and hydrogen sulphid are produced; optimum temperature about 30° C., maximum 36°, minimum below 2°; thermal death point near 50°. Sensitive to sodium chlorid and to acids; readily killed by drying and by exposure to sunlight. Gram-negative; not acid-fast. Group number 211.2322523.

Pathogenic on leaves of gladioli forming more or less angular, translucent spots.

Type specimens have been deposited in the herbarium of the Bureau of Plant Industry, United States Department of Agriculture.

## SUMMARY

The bacterial disease described in this paper is capable of causing serious injury to the leaves of the gladiolus and consequently it interferes with the development of the corms.

The lesions are more or less angular, translucent spots. From the infected tissues there is a rather copious and viscid exudate which when dry forms a thin, brittle layer, or small drops over the surface. The leaves are often coated with soil particles which have become embedded in the exudate.

The bacteria are very abundant in the infected areas and pure cultures are easily isolated. On culture media this bacterium produces a yellow, viscid growth. It is motile, having one flagellum; capsules are present; no spores. It is Gram-negative and not acid-fast. The group number is 211.2322523.

Numerous inoculations resulting in successful infections have proved that the bacterium isolated from the leaves is the cause of the disease.

The organisms gain entrance to the tissues by way of the stomata. They invade the parenchyma and fill the intercellular spaces and cavities resulting from destruction of cell walls.

PLATE 1

*Bacterium gummisudans* on gladiolus

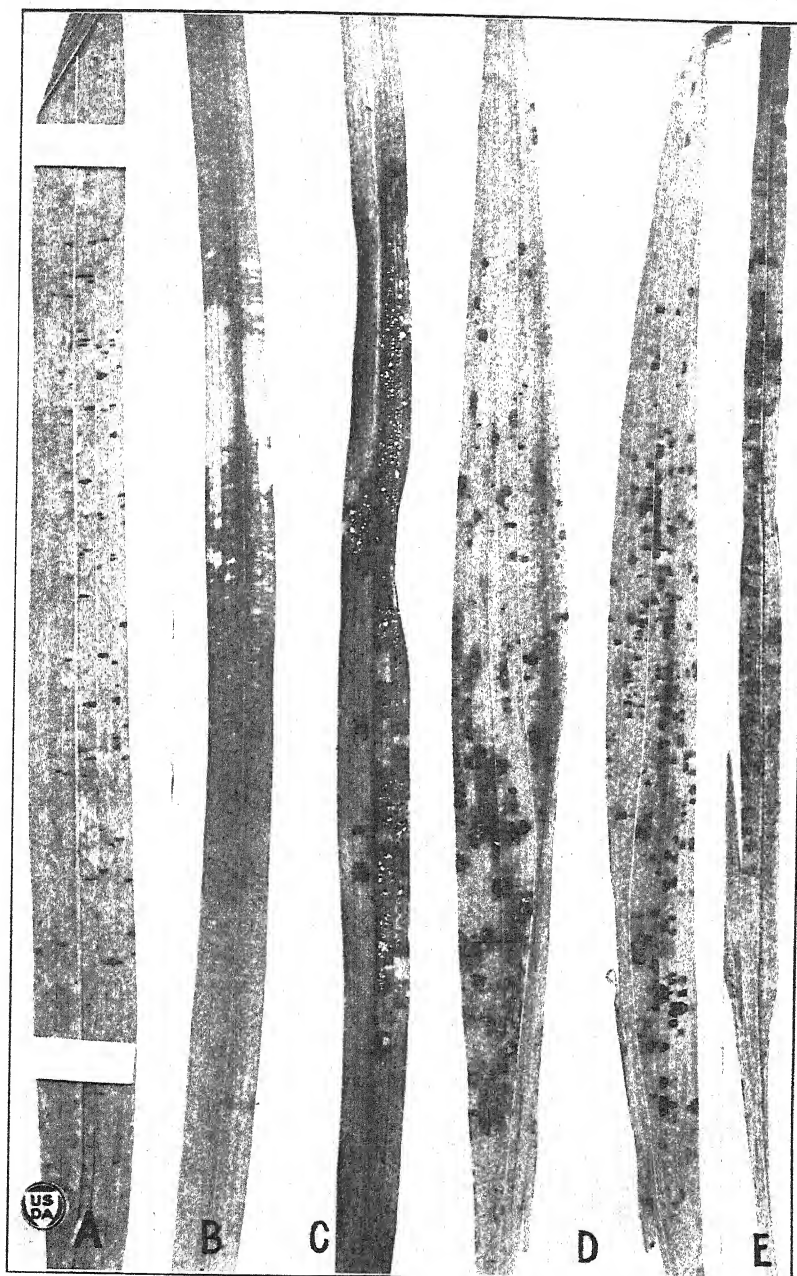
A.—Early stage of the bacterial blight, showing the water-soaked, horizontal lesions. Natural infection.  $\times 1$ .

B.—Artificial inoculation 18 days after inoculation. Photographed by transmitted light.  $\times 1$ .

C.—Artificial inoculation showing the dry exudate on the lesions.  $\times 1$ . A, B, and C are leaves from "seedling" plants.

D.—Leaves from blooming plants (var. Schwaben) showing general appearance in the field. Natural infections.  $\times 1/2$ .

E.—Leaf from seedling plant (var. Schwaben). Natural infection.  $\times 1$ .



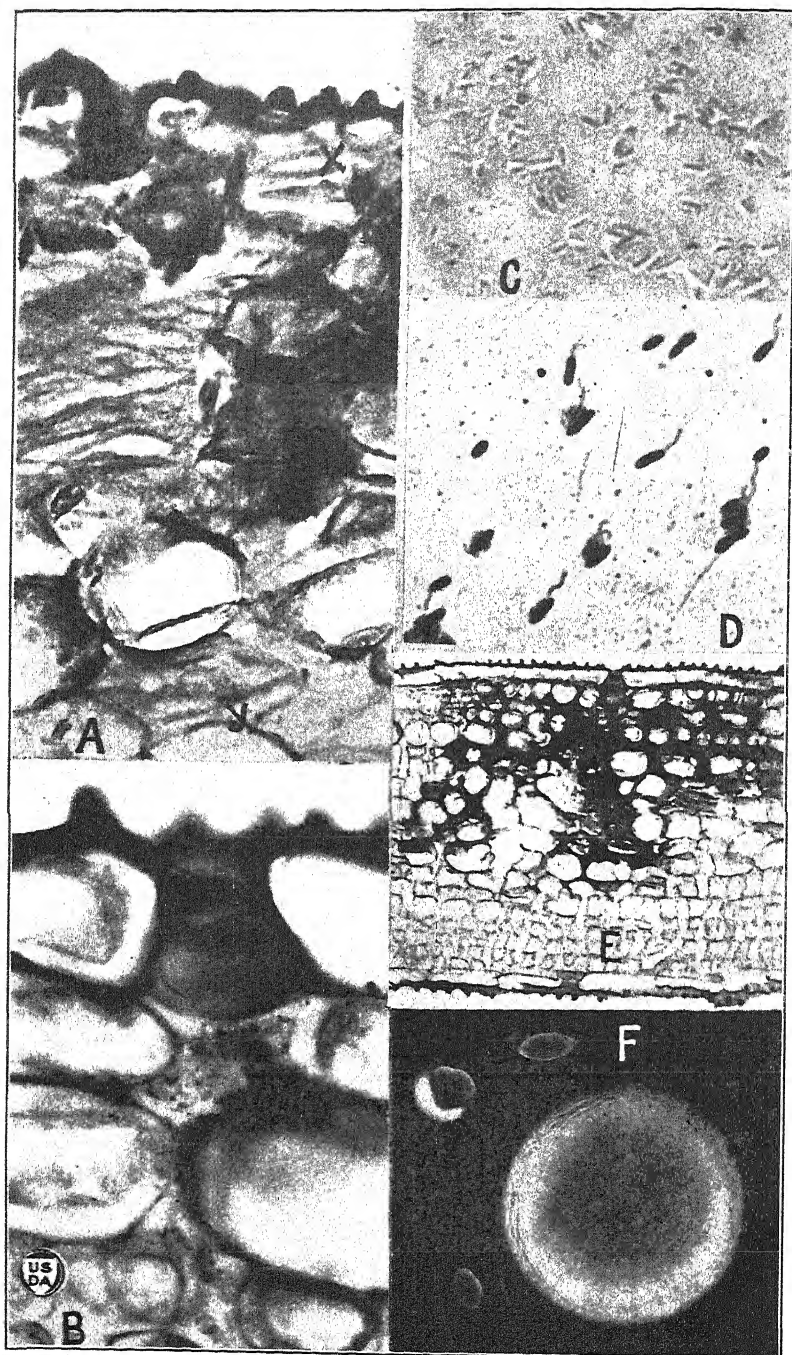




PLATE 2

*Bacterium gummisudans* on gladiolus

A.—Lesions showing bacteria arranged in layers in the central cavity and also in the cells  $x$  and  $y$  where a part, at least, of the cell wall still exists.

B.—Early stage of stomatal infection. Only a few bacteria are in the stomatal chamber and the adjoining intercellular spaces.  $\times$  about 1,000.

C.—Capsules. Bacteria from a 4-day beef agar culture.  $\times 2,000$ .

D.—Flagella. Bacteria from 2-day-old beef agar culture. Casares-Gil's stain. Bacteria are viscid and it is difficult to get an even distribution of single rods.  $\times 2,500$ .

E.—Later stage than B. Bacteria in great numbers have penetrated widely into the intercellular spaces but the cell walls are as yet only slightly affected.

F.—Surface and buried colonies on beef agar plate (3 weeks old). The surface colony shows the characteristic striations.  $\times 5$ .

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No. 5

## THE BLOOMING OF WHEAT FLOWERS<sup>1</sup>

By C. E. LEIGHTY and W. J. SANDO, *Agronomists, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture*

### INTRODUCTION

The time and manner of blooming of wheat flowers is the result of the interaction of internal and external factors. When flowers have reached the blooming stage, the exact time and rate of blooming is determined to a large extent by meteorological conditions.

The observations on wheat flowers reported in this paper were made primarily for the purpose of procuring additional information on the effect of external factors on blooming.

### METHODS AND MATERIALS

The plants on which most of the observations were made were volunteers growing in a garden in Washington, D. C. They were of the Fultz type (*Triticum vulgare*), having the following characters: Plant, winter habit; spike, awnless; glumes, glabrous, white; kernels, red, soft. More than 400 flowers in seven heads on four plants, which began to bloom at about the same time, were chosen for observation. Additional observations were made on plants growing in the field and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va.

A plane view diagram of a wheat head illustrated by figure 1 shows the method used for recording the data. In figure 1, the rectangular spaces inclosed by solid lines represent the odd-numbered spikelets found on one side of a head, numbering from the base of the head, and the rectangular spaces inclosed by dotted lines represent the even numbered spikelets found on the opposite side. The spikelet number is shown within the large square located in the middle of each rectangular space. The numbers in the small squares adjoining the large square indicate the alternate position of each flower on the spikelet from the lowest to the highest. The exact time of blooming was recorded in the spaces opposite the flower number.

Flowers blooming cleistogamously were recorded as "dehisced inside." Blank spaces indicate sterile flowers. On spikelets 6, 7, 12, and 13, of head No. 4A (see fig. 1), fifth flowers bloomed but were not included in the data.

The seven heads chosen were watched continuously for six days, with the exception of a short period each night between 10 p. m. and 1.55 a. m. when little active blooming occurred, and the exact time of blooming of practically all flowers was recorded. Observations at night were made with the aid of a small electric flash light which was directed on the flowers at various intervals long enough only to procure the necessary information for record.

<sup>1</sup> Received for publication, Dec. 13, 1923.

6:15 P.M. $\frac{9}{15}$	4 2	22	3 1	323 A.M. $\frac{9}{10}$ 4:50 P.M. $\frac{9}{14}$
1:35 A.M. $\frac{9}{17}$	3	21	4	
6:09 P.M. $\frac{9}{14}$	1		2	7:42 A.M. $\frac{9}{16}$
8:15 P.M. $\frac{9}{16}$	3	20	4	
4:45 P.M. $\frac{9}{14}$	1		2	4:47 P.M. $\frac{9}{15}$
10:23 A.M. $\frac{9}{10}$	4	19	3	5:32 P.M. $\frac{9}{16}$
4:19 P.M. $\frac{9}{15}$	2		1	DEHISCED INSIDE
2:43 P.M. $\frac{9}{16}$	3	18	4	4:08 P.M. $\frac{9}{10}$
DEHISCED INSIDE	1		2	1:47 P.M. $\frac{9}{15}$
4:00 A.M. $\frac{9}{17}$	3	17	4	
12:10 P.M. $\frac{9}{16}$	1		2	12:45 P.M. $\frac{9}{15}$
2:25 P.M. $\frac{9}{16}$	3	16	4	323 A.M. $\frac{9}{10}$
9:10 A.M. $\frac{9}{15}$	1		2	10:26 P.M. $\frac{9}{15}$
11:30 A.M. $\frac{9}{17}$	4	15	3	9:35 A.M. $\frac{9}{16}$
DEHISCED INSIDE	2		1	9:38 P.M. $\frac{9}{15}$
DEHISCED INSIDE	3	14	4	11:30 A.M. $\frac{9}{17}$
9:59 A.M. $\frac{9}{16}$	1		2	9:30 A.M. $\frac{9}{15}$
9:35 P.M. $\frac{9}{16}$	4	13	3	Between 8:30 P.M. $\frac{9}{10}$ and 9:35 P.M. $\frac{9}{10}$
9:38 P.M. $\frac{9}{15}$	2		1	8:55 A.M. $\frac{9}{16}$
10:28 P.M. $\frac{9}{16}$	3 1	12	4	11:59 A.M. $\frac{9}{17}$
12:06 P.M. $\frac{9}{17}$	4	11	3	10:26 A.M. $\frac{9}{15}$
12:28 P.M. $\frac{9}{15}$	2		1	Between 9:30 P.M. $\frac{9}{10}$ and 2:55 A.M. $\frac{9}{17}$
3:22 P.M. $\frac{9}{16}$	3	10	4	4:50 A.M. $\frac{9}{19}$
5:33 P.M. $\frac{9}{14}$	1		2	4:34 P.M. $\frac{9}{15}$
4:30 P.M. $\frac{9}{16}$	3	9	4	4:09 A.M. $\frac{9}{10}$
8:12 A.M. $\frac{9}{15}$	1		2	6:08 P.M. $\frac{9}{15}$
7:10 P.M. $\frac{9}{16}$	3	8	4	8:05 A.M. $\frac{9}{10}$
11:10 A.M. $\frac{9}{15}$	1		2	8:54 A.M. $\frac{9}{16}$
10:16 P.M. $\frac{9}{10}$	4	7	3	11:30 A.M. $\frac{9}{17}$
10:02 P.M. $\frac{9}{16}$	2		1	3:46 P.M. $\frac{9}{15}$
2:33 P.M. $\frac{9}{10}$	4	6	3	4:08 A.M. $\frac{9}{10}$
3:03 P.M. $\frac{9}{16}$	2		1	Between 10:05 P.M. $\frac{9}{15}$ and 2:45 A.M. $\frac{9}{16}$
5:25 A.M. $\frac{9}{10}$	3	5	4	Between 3:30 P.M. $\frac{9}{10}$ and 3:55 A.M. $\frac{9}{10}$
8:30 A.M. $\frac{9}{16}$	1		2	4:30 P.M. $\frac{9}{16}$
12:30 P.M. $\frac{9}{10}$	4	4	3	10:52 A.M. $\frac{9}{10}$
11:40 A.M. $\frac{9}{17}$	2		1	10:08 A.M. $\frac{9}{16}$
5:35 P.M. $\frac{9}{10}$	3	3	4	6:30 A.M. $\frac{9}{10}$
5:06 P.M. $\frac{9}{16}$	1		2	4:00 A.M. $\frac{9}{10}$
11:25 A.M. $\frac{9}{10}$	3	2	4	
11:51 A.M. $\frac{9}{17}$	1		2	7:29 A.M. $\frac{9}{10}$
DEHISCED INSIDE	4 2	1	3 1	DEHISCED INSIDE 12:05 P.M. $\frac{9}{17}$

FIG. 1.—Diagram of wheat head No. 4A, showing the method used for recording the data on time of blooming of the different flowers.

Immediately after recording the time of blooming of a flower, the empty extruded anthers were removed with forceps in order to facilitate observations and to prevent confusion in recording the data. Great care was required in this procedure since the slightest jarring or touching of a head while blooming is likely to hasten the opening of the flowers. A flower was considered as having bloomed as soon as noticeable separation of the lemma from the palea occurred.

#### THE BLOOMING PROCESS

Under favorable conditions the wheat flower blooms by opening its glumes, slowly at first, then more rapidly, until the tips of the lemma and palea are separated, usually 3 or 4 mm. After the glumes open the anthers are pushed upward by the elongating filament and when fully extruded assume a pendent position. At some time during these processes the anthers dehisce apically along the lines joining the cylinders into pairs. This splitting may take place in one or more anthers by the time they are first clearly visible, while in others it may be delayed until the time of full extrusion. A number of flowers were observed whose anthers were completely extruded and pendent before dehiscence occurred. Some flowers were found which had retained within their glumes one, two, or three anthers, quite often lodged in the folds of the palea. Other flowers were found with anthers partially protruded and imprisoned between the tips of the glumes. The greatest length of filament observed was 10 mm., exclusive of the anther, which was 3 mm. long. In one instance a filament was observed to attain its full length of 10 mm. in 10 minutes. Askenasy (*1*)<sup>2</sup> measured a number of filaments of wheat and rye flowers. He states that in most cases the filaments have grown from 1 to 1½ mm. per minute.

Very soon after full extrusion, and assisted by the inversion of the anthers, the pollen is fully emptied from the anther sacs. Some of it, probably at least one-third, usually falls within its own flower, resulting in plentiful pollination of the stigma. The remaining pollen is scattered about and may fall upon the stigmas of other flowers. Cross pollination apparently is sometimes effected in this way, especially when the anthers of neighboring flowers have been removed, as shown by Leighty and Hutcheson (*8*), or have aborted.

When conditions are unfavorable for the opening of the glumes, the anthers of the wheat flower shed their pollen and effect fertilization without being extruded, or they may be extruded only at the tips. Nearly 5 per cent of the flowers, or 19 out of a total of 406, under observation in this study, behaved this way. Each of the heads under observation had one or more such cleistogamous flowers, the maximum number on any head being six. Kernels were procured from all of these flowers. Under certain environmental conditions wheat flowers may be quite generally cleistogamous.

#### TIME REQUIRED

The process of blooming of wheat flowers is variable. Some flowers have been observed to open in less than 1 minute. Others require 3 minutes or more. Observations on the time elapsing between the beginning of opening and the time at which the anthers attain the

<sup>2</sup> Reference is made by number (*italic*) to "Literature cited," p. 244.

pendent position following their complete extrusion from the glumes were made on 25 flowers at different hours of the day favorable for blooming. The time required for the anthers to assume the pendent position varied in these 25 flowers from 1 minute 40 seconds to 5 minutes 25 seconds, and averaged 3 minutes 36 seconds. The time required depends on (1) the position of the head at the time of the protrusion of the anthers, (2) the resistance offered by the glumes, (3) air movement, (4) temperature, and (5) rainfall and humidity.

The time elapsing between the opening and closing of the glumes of a flower was found to vary from 11 to 66 minutes. Frequently difficulty was experienced in determining the exact time when the glumes were closed completely, for sometimes anthers or filaments became lodged obscurely between the glumes in such a way that complete closing was prevented. Table I shows the duration of blooming of 25 flowers, with their location on the spike. The average duration was found to be 26.5 minutes.

TABLE I.—*Time of opening and closing and time elapsing between opening and closing of 25 flowers, with head number, position of spikelet on the head and position of the observed flowers on the spikelet*

Head number.	Spikelet number.	Flower number.	Time of opening.	Time of closing.	Time between opening and closing.
			1922	1922	Minutes.
2 D.....	17	1	5.55 p. m., May 14...	6.18 p. m., May 14...	23
3 C.....	10	1	5.28 p. m., May 14...	5.41 p. m., May 14...	13
3 C.....	16	1	5.29 p. m., May 14...	5.40 p. m., May 14...	11
4 A.....	22	1	6.15 p. m., May 15...	6.36 p. m., May 15...	21
4 A.....	22	2	4.50 p. m., May 14...	5.20 p. m., May 14...	30
4 A.....	17	2	12.45 p. m., May 15...	1.10 p. m., May 15...	25
4 A.....	11	2	12.28 p. m., May 15...	12.50 p. m., May 15...	22
4 A.....	12	2	10.26 a. m., May 15...	10.45 a. m., May 15...	19
4 A.....	20	1	4.43 p. m., May 14...	5.10 p. m., May 14...	27
4 A.....	10	1	5.33 p. m., May 14...	6.15 p. m., May 14...	42
5 C.....	15	1	5.07 p. m., May 14...	5.40 p. m., May 14...	33
5 C.....	11	2	2.25 p. m., May 15...	2.45 p. m., May 15...	20
5 C.....	13	1	5.07 p. m., May 14...	5.40 p. m., May 14...	33
5 C.....	5	1	1.39 p. m., May 16...	2.15 p. m., May 16...	36
5 C.....	8	2	8.21 a. m., May 16...	8.50 a. m., May 16...	29
5 C.....	14	1	5.30 p. m., May 14...	5.50 p. m., May 14...	20
6 C.....	13	1	5.30 p. m., May 14...	5.55 p. m., May 14...	25
7 D.....	7	1	7.49 a. m., May 16...	8.55 a. m., May 16...	66
7 D.....	18	2	8.34 a. m., May 16...	9.15 a. m., May 16...	41
8 B.....	17	1	10.27 a. m., May 15...	10.45 a. m., May 15...	18
8 B.....	13	1	9.30 a. m., May 15...	10.00 a. m., May 15...	30
8 B.....	16	1	10.27 a. m., May 15...	10.48 a. m., May 15...	21
8 B.....	12	1	8.13 a. m., May 15...	8.29 a. m., May 15...	16
8 B.....	10	1	10.27 a. m., May 15...	10.48 a. m., May 15...	21
8 B.....	8	1	10.27 a. m., May 15...	10.48 a. m., May 15...	21
Average.....					26.5

Kirchner (5) and Koernicke and Werrier (7) state that under favorable conditions wheat flowers remain open for about a quarter of an hour. According to Knuth (6) both Rimpau and Delpino found that each flower remains open only about 15 minutes. Fr  wirth (2) states that the flower is closed usually in 12 to 20 minutes, sometimes 8 to 35 minutes, from the beginning of opening. Obermayer (9) records the duration of

blooming under most favorable conditions as 13 to 18 minutes, but states that it sometimes is completed in shorter periods, even as low as 2 or 3 minutes, while under less favorable conditions and in the early morning it requires from 25 to 32 minutes. The average duration is 22 minutes.

Percival (10, *p.* 122-129) states that the time taken in opening and closing completely varied from 8 to 30 minutes or more, the average being 20 minutes, while Hays (4, *p.* 50) says, "The floret (of wheat) usually opens about dawn, and closes again within an hour."

#### PERIOD OF BLOOMING

Flowers were observed blooming during various hours of daylight and darkness. "Day" is hereafter referred to as a 24-hour period; "daylight," the period from sunrise to sunset; "twilight," the combined periods of approximately  $1\frac{3}{4}$  hours each before sunrise and after sunset. "Night" includes the remaining portion of a day not previously designated. The division of the day into these periods was based on the time of sunrise and sunset (Table II) kindly supplied by the United States Naval Observatory at Washington, D. C.

Of the 406 flowers observed, 6.9 per cent bloomed at night; 6.9 per cent during twilight, and 86.2 per cent in the daylight. The total number of flowers blooming on each head and the numbers blooming in each period of the day during the complete period of anthesis are shown in Table III.

TABLE II.—*Time of rising and setting of the sun at Washington, D. C., May 14 to 19, 1921*

	May 14.	May 15.	May 16.	May 17.	May 18.	May 19.
Sunrise, a. m. ....	4.56	4.55	4.54	4.54	4.53	4.52
Sunset, p. m. ....	7.12	7.13	7.14	7.15	7.16	7.17

TABLE III.—*Total number of flowers blooming and numbers blooming during stated periods of the day, during the complete period of anthesis, on each of 7 heads of wheat*

Head No.	Number of flowers blooming.			
	Total.	In daylight.	In twilight.	In night.
2 D. ....	59	52	4	3
3 C. ....	52	42	4	6
4 A. ....	75	62	9	4
5 C. ....	41	37	1	3
6 C. ....	51	47	2	2
7 D. ....	53	47	3	3
8 B. ....	75	63	5	7
Total. ....	406	350	28	28
Per cent. ....	100	86.2	6.9	6.9

The fact that most of the flowers under observation bloomed in daylight does not mean, necessarily, that light is essential for blooming. As shown elsewhere in this paper, wheat flowers kept in a dark room bloomed completely and in the same manner as flowers under natural conditions. Although no data are at hand, it is probable that night

blooming might be considerably more abundant than it was in this experiment under certain environmental conditions.

The effect on blooming of the duration of the daily illumination period, or of changes in the duration of this period, was not determined. It is known from other studies, however, that many varieties of wheat will bloom and mature in less time after planting when subjected to a long daily illumination period as compared with a shorter period.

According to Godron (3) wheat flowers open at 4.30 a. m. when the temperature is at least 16° C., with blooming ending at 6.30 to 7 o'clock. Rimpau (11) states that under favorable conditions blooming begins at 4.30 a. m. and occurs during various periods of the day. He reports that he observed open flowers after 6.45 p. m., but does not mention the latest hour at which blooming was observed. Koernicke (7) states that open flowers may be observed at all hours of the day, the earliest around 5.45 a. m., the latest at 8.30 p. m. Frürwirth (2) observed flowers blooming during the day from 4.30 a. m. to 7 p. m. under favorable conditions. Obermayer (9) found that blooming began even before 5 a. m. and continued throughout the day, with a small number blooming after 7 p. m. Salmon (12), describing conditions in western South Dakota, says: "It is exceptional to find wheat in bloom after 7 a. m. under normal conditions."

#### ORDER OF BLOOMING

Table IV shows the positions on the head of the first five flowers blooming on each of the seven heads studied. On five of the seven heads the first flower bloomed in the lower half of the uppermost third of the head; on one head, in the upper half of the middle third; and on another head, in the upper half of the uppermost third.

Frürwirth (2) states that the first blooming occurs between the middle of the head and the top of the middle third. Percival (10, p. 122-129) says that the position of the first flower to bloom is generally in the middle third of the head, usually in the upper part. Godron (3) found that the first flower bloomed below the upper third of the head. Rimpau (11) states that the first flower began to bloom at a point two-thirds to three-fourths of the distance from the base of the head.

Table V shows the position of the last flower observed to bloom on each head. On heads not included in this study flowers in the terminal spikelet have occasionally been seen blooming before those in spikelets located in the upper third of the head.

TABLE IV.—*Number of spikelets in each of seven wheat heads and position of the spikelets, numbered in order from the base of the spike, in which were located the first five and the last blooming flowers, with flower number of last flower*

Head No.	Total number of spikelets.	Position number of spikelets where occurred blooming of first five and last flowers.						Number of last flower.
		1st.	2d.	3d.	4th.	5th.	Last.	
2 D.....	20	16	14	15	20	17	5	4
3 C.....	18	13	15	14	12	11	3	3
4 A.....	22	20	22	10	21	9	3	4
5 C.....	18	12	15	13	11	14	9	3
6 C.....	19	16	14	11	12	13	4	3
7 D.....	21	15	17	14	13	16	8	3
8 B.....	23	15	12	11	13	9	2	2



Considering the head as a whole, the flowers above the first flower to bloom completed their blooming before those situated below this flower. From 8 to 60 per cent of the flowers below remained unopened at the time those above had finished blooming.

The lowest flower in the spikelet usually blooms first, and the other flowers of the spikelet bloom in order from lowest to highest on successive days. A few exceptions were found, however, where flowers failed to behave in this manner (see fig. 1, head 4A, spikelets 11, 12, 13, and 17). A number of instances were found where two flowers in the same spikelet bloomed on the same day. Complete data on these flowers is shown in Table V. In all, 39 such instances were recorded. The average time which elapsed between the blooming of the two flowers in the spikelet was nine hours and four minutes.

TABLE V.—Location, by spikelet and flower number, of two flowers in the same spikelet which bloomed on the same day, with the hour of each blooming and the interval between these bloomings

Head No.	Spikelet No.	Flower number and hour of blooming.				Interval between bloomings.
		1st.	2d.	3d.	4th.	
2 D	5	8.45 a. m. ....	8.30 p. m. ....	.....	.....	11 45
	6	7.52 a. m. ....	2.42 p. m. ....	.....	.....	6 50
	6	.....	.....	8.27 a. m. ....	5.45 p. m. ....	9 13
	10	.....	8.20 a. m. ....	9.15 p. m. ....	.....	12 55
	11	8.43 a. m. ....	3.45 p. m. ....	.....	.....	7 02
	18	6.45 a. m. ....	6.12 p. m. ....	.....	.....	11 27
3 C	8	10.16 a. m. ....	7.55 p. m. ....	.....	.....	9 39
	9	9.15 a. m. ....	4.29 p. m. ....	.....	.....	7 14
4 A	5	8.30 a. m. ....	4.30 p. m. ....	.....	.....	8 ..
	6	4.08 a. m. ....	2.33 p. m. ....	.....	.....	10 25
	8	.....	8.56 a. m. ....	7.10 p. m. ....	.....	10 14
5 C	9	8.12 a. m. ....	6.08 p. m. ....	.....	.....	9 56
	16	9.10 a. m. ....	10.26 a. m. ....	.....	.....	1 16
	6	8.30 a. m. ....	3.13 p. m. ....	.....	.....	6 43
	8	.....	8.21 a. m. ....	9.40 p. m. ....	.....	13 19
6 C	10	6.49 a. m. ....	3.35 p. m. ....	.....	.....	8 46
	16	8.24 a. m. ....	6.45 p. m. ....	.....	.....	10 21
	6	9.23 a. m. ....	5.10 p. m. ....	.....	.....	7 47
	9	.....	7.56 a. m. ....	8.28 p. m. ....	.....	12 32
7 D	10	9.18 a. m. ....	3.53 p. m. ....	.....	.....	6 35
	13	.....	.....	3.09 p. m. ....	2.35 p. m. ....	11 26
	3	4.47 a. m. ....	8.30 p. m. ....	.....	.....	15 43
	6	8.49 a. m. ....	5.07 p. m. ....	.....	.....	8 18
8 B	7	7.49 a. m. ....	4.17 p. m. ....	.....	.....	8 28
	12	11.12 a. m. ....	8.31 p. m. ....	.....	.....	9 19
	13	.....	7.34 a. m. ....	5.07 p. m. ....	.....	9 33
	14	7.51 a. m. ....	5.43 p. m. ....	.....	.....	9 52
	16	8.02 a. m. ....	4.52 p. m. ....	.....	.....	8 50
	5	8.09 a. m. ....	5.03 p. m. ....	.....	.....	8 54
8 B	6	9.23 a. m. ....	3.50 p. m. ....	.....	.....	6 27
	8	.....	9.44 a. m. ....	8.26 p. m. ....	.....	10 42
	9	9.30 a. m. ....	6.17 p. m. ....	.....	.....	8 47
	10	10.27 a. m. ....	5.15 p. m. ....	.....	.....	6 48
	11	9.30 a. m. ....	5.15 p. m. ....	.....	.....	7 45
	12	8.13 a. m. ....	2.38 p. m. ....	.....	.....	6 25
	13	9.30 a. m. ....	5.15 p. m. ....	.....	.....	7 45
	14	10.20 a. m. ....	5.15 p. m. ....	.....	.....	6 55
	16	10.27 a. m. ....	6.48 p. m. ....	.....	.....	8 21
	21	9.42 a. m. ....	8.50 p. m. ....	.....	.....	11 08

Percival (10, p. 122-129) states that the lowest flower of a spikelet blooms first, with the others following on successive days. Fr  wirth (2) notes the exception that the second flower, instead of blooming on the next succeeding day, may sometimes bloom in the afternoon of the first day, while the remaining flowers may bloom on the following day or on the second day after the blooming of the second flower.

More than half of the first or lowest flowers in all the spikelets on the seven heads under observation were observed to bloom before the second flower in any spikelet bloomed (Table VI), and about 85 per cent of the first flowers bloomed before any flower in the third position bloomed. Nearly two-thirds of the second flowers in turn bloomed before the third flower in any spikelet bloomed, and a similar relationship exists in the blooming time of the third and fourth flowers. If the earliest blooming of second, third, and fourth flowers in any spikelet on a head is considered as marking off periods of blooming for that head, it is found that a part of the first or lowest flowers bloom subsequently to the opening of the earliest of the third flowers and even the earliest of the fourth flowers. Similarly, some of the second and third flowers will bloom after the first blooming of third and fourth flowers, respectively. The data on these periods in the heads under observation are shown in Table VI.

TABLE VI.—*Relative period of blooming of the first to fourth flowers in the spikelets of seven wheat heads*

Relative period of blooming.	Percentage of flowers in each position blooming in each period.				
	1st.	2d.	3d.	4th.	All.
Previous to the first blooming of a second flower.....	<i>Per cent.</i> 54.13	<i>Per cent.</i> .....	<i>Per cent.</i> .....	<i>Per cent.</i> .....	<i>Per cent.</i> 17.74
From the blooming of the earliest second flower to the blooming of the earliest third flower.....	30.83	64.40	.....	<sup>a</sup> 1.79	29.06
From the blooming of the earliest third flower to the blooming of the earliest fourth flower.....	10.53	27.97	69.70	.....	28.57
Subsequent to the blooming of the earliest fourth flower.....	4.51	7.63	30.30	98.21	24.63
	100.00	100.00	100.00	100.00	100.00

<sup>a</sup> One flower only; disregarded in period determinations.

The numbers of flowers that bloomed at the different hours of the day and night throughout the entire blooming periods of the seven wheat heads are shown in Table VII. Since the actual time of blooming of flowers was not observed between 10 p. m. and 1.55 a. m., all of the flowers blooming between these hours are grouped and recorded as of this period. The maximum number of flowers blooming on a single head in one day was 25. This occurred on May 16, one of the most active blooming days in the period of observation. Obermayer (9) observed a maximum of 23 flowers on one head opening in one day.

The maximum number of flowers blooming on a single head in one hour in this study was found to be eight, these being divided equally between the two sides of the head. The blooming on all heads was completed within 101 to 123 hours.

TABLE VII.—Number and distribution per head of wheat flowers observed in the actual process of blooming between stated hours on each day of the period of anthesis at Washington, D. C., 1922.

Blooming period.	May 14.			May 15.				May 16.				May 17.				May 18.				May 19.				Hourly total.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
	Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.	Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.	Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.		All heads.	Head 2.	Head 3.	Head 4.	Head 5.	Head 6.	Head 7.	Head 8.	All heads.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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## METEOROLOGICAL RELATIONS

The meteorological data used in this study are those for Washington, D. C., and were procured from the United States Weather Bureau. That temperature, rainfall, and sunshine are intimately associated with the blooming of wheat flowers appears evident from a consideration of figure 2. Temperature, however, seems to be the most important of the meteorological influences affecting blooming. The two days showing the most significant relation between temperature and flower blooming are May 15 and May 16, when almost perfect sunshine with no rainfall was registered. On these two days 60.8 per cent of all the flowers bloomed. Maximum blooming occurred on May 15 and 16, when the temperatures were 76° and 78° F., respectively. Active blooming usually begins shortly after sunrise, on days with clear skies, and anthers in flowers opening on these days dehisce more rapidly than those in flowers opening on cloudy days. Cloudiness or rain has the effect of retarding blooming, principally through the lowering of the temperature to a point below the optimum for this process. On cloudy or rainy days, however, when the temperature is favorable for blooming the flowers either open incompletely or bloom cleistogamously. On May 18 flowers were observed opening in a drizzling rain and pollen was shed from the anthers. No flowers were observed to open at any time during a rain of such intensity as to permit droplets of water to adhere to the glumes.

Three periods of intense blooming were noted: (1) On May 14 between 5 and 6 p. m., (2) on May 15 between the hours of 9 to 10 a. m., 1 to 2 p. m., and 3 to 4 p. m., and (3) on May 16 between the hours of 9 to 10 a. m., 1 to 3 p. m., and 5 to 6 p. m. The weather conditions on May 15 and 16 were very similar, and it is interesting to note the consistent correspondence of the three periods of intense blooming on these two days. The single period of intense blooming on May 14 apparently corresponds to the third period on May 15 and 16. On both the latter days a larger number of flowers bloomed in the second or middle period than in the first or third periods, which are of approximately the same intensity. The interval between the first and second blooming periods of these two days is approximately twice as great as between the second and third periods.

Früwirth (2), of Austria, states that when the temperature at 4.30 a. m. is above 14° C. the blooming begins at this hour. Many flowers bloom from this time to 5.30, while from 5.30 to 9 the number is less. Very many bloom from 9 to 10 a. m., while very few bloom from 10 a. m. to 2.30 p. m. From 2.30 to 3.30 p. m. the number again is large, while from 3.30 to 7 p. m. it again is small. The principal blooming time of the morning, made up of a preceding and succeeding period of which the first or the second may be the stronger, is followed in the afternoon by a later blooming period which approaches in intensity the weaker part of the morning blooming time.

On May 17, when lower temperatures prevailed, the only period of intense blooming was between 11 a. m. and 12 m., while on May 18 two periods occurred, one from 3 to 4 a. m. and one from 10 to 11 a. m. The intense blooming on May 14, 15, and 16 exhausted the flowers available for blooming to such an extent that with unfavorable atmospheric conditions on the 17th and 18th (cloudiness, rainfall, fluctuating temperature) only irregular blooming occurred on these days.

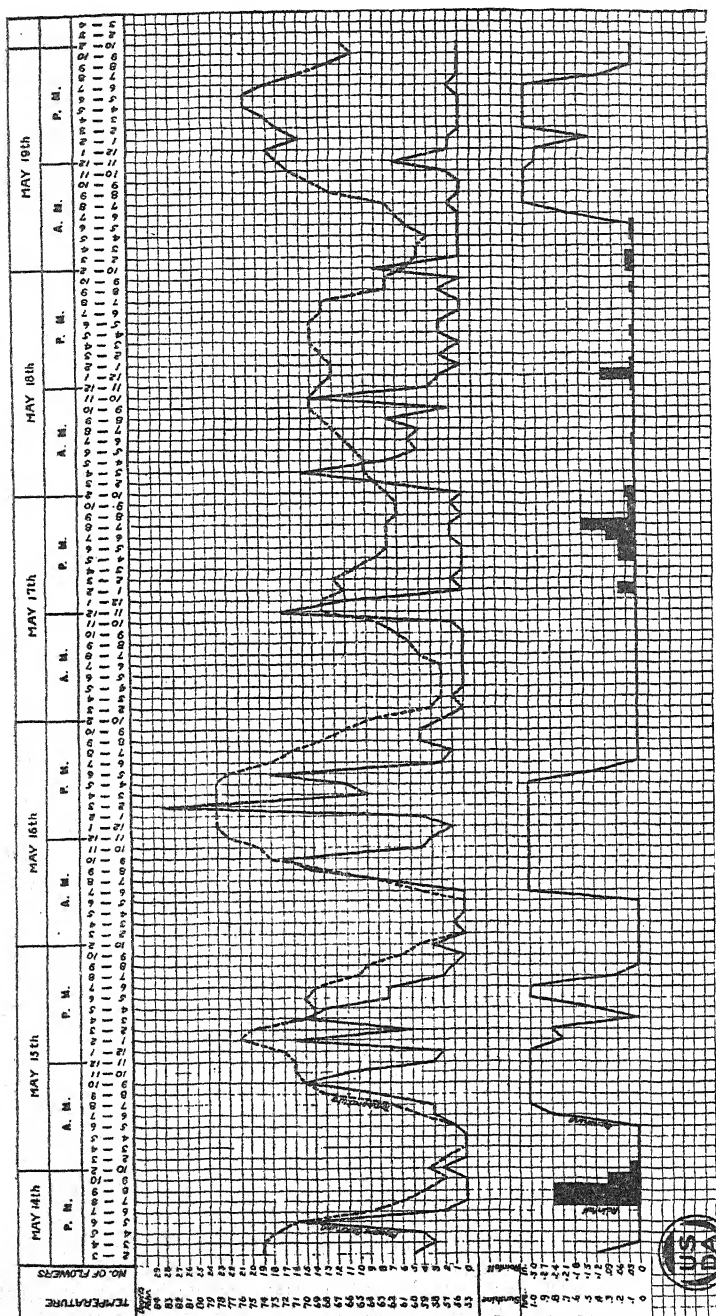


FIG. 2.—Temperature, rainfall, and sunshine record and number of flowers blooming on seven wheat heads at different hours in the period from 2 p. m., May 14, to 10 p. m., May 19, 1922, at Washington, D. C.

Obermayer (9), in Germany, reports that the most active blooming occurs in the early morning from before 7 a. m. to 11 a. m., followed by a marked decrease after 11 a. m., and reaching the lowest point between 1 and 3 p. m. From 3 to about 7 p. m. blooming again increases, but reaches only about 60 per cent of the intensity of that between 7 and 11 a. m.

Percival (10. *p.* 122-129), at Reading, England, observed intense blooming periods between 5 and 7 a. m., 9 and 10 a. m., 2 and 3 p. m., and 8 and 9 p. m. Shitkova (13), at Saratov, Russia, noted two active blooming periods at 5 to 7 a. m. and 5 to 6 p. m. Leighty and Hutcheson (8), at two different places in the United States, observed two intense blooming periods, one from 7 to 9 a. m. and another in the middle of the afternoon.

The minimum temperature recorded during the whole experiment was 55° F., while the maximum temperature was 78° F. The lowest temperature at which blooming occurred was 56° F.; the highest, 78° F.

Godron (3) states that blooming begins at 4.30 a. m. when the temperature is not below 16° C. Frürwirth (2) says that blooming begins at 4.30 a. m. when the temperature is above 14° C. Rimpau (11) and Koernicke (7) found the minimum temperature at which flowers bloom to be 12° and 13° C., respectively. Obermayer (9) observed blooming in a single strain of wheat toward 6 a. m. at 14° C. in a thick mist and toward 7 a. m. at 15° C. in a mist and dropping rain.

Of all the flowers which bloomed on six of the heads under observation 80.2 per cent set seed, as is shown in Table VIII. The seventh head was destroyed by birds before harvest. The percentage of flowers setting seed in the four different positions in the spikelet, is largest for the first or lowest position, nearly all of these flowers producing kernels. The percentage decreases from the lowest to the highest position, little difference existing, however, between those in the first and second positions.

#### ADDITIONAL OBSERVATIONS

The following observations were made separately from those reported in the preceding part of this paper: (1) Wheat heads on plants grown in the field, which were completely submerged in water from several days before blooming until complete maturity of the heads, were found to open their flowers apparently in the same manner as under normal, unaltered conditions, with the exception that the filaments did not elongate nor did the anthers dehisce. Flowers under these circumstances remained open for several days.

(2) Plants were placed in a light-tight (dark) room at a temperature between 60° to 70° F. for one week at the time of blooming. The flowers on the heads bloomed completely under these conditions in approximately the same length of time as under normal conditions. Flowers were observed with glumes as widely separated in the dark as in the daylight, under favorable temperature conditions.

(3) In a greenhouse at a temperature of 55° to 56° F. flowers were observed with their glumes separated 3 mm. (The greatest separation under normal conditions was found previously to be 4 mm.) Movement of the anthers was easily observed at this temperature. When the temperature was gradually lowered, extension of the filament was retarded, so that at a temperature below 55° F. no perceptible further movement

was detected, and the anthers appeared to remain stationary, but pollen was discharged at as low a temperature as 52° F.

The maximum temperature at which flowers were observed opening in a greenhouse was 80° F.

TABLE VIII.—Numbers of flowers blooming and kernels produced in different positions in the spikelets on six wheat heads

Head No.	Location in spikelet.									
	1st flower.		2d flower.		3d flower.		4th flower.		Total.	
	Number of—		Number of—		Number of—		Number of—		Number of—	
	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.	Flowers blooming.	Kernels obtained.
2 D.....	18	17	18	18	16	14	9	5	61	54
3 C.....	17	16	17	17	15	12	7	3	56	48
4 A.....	22	22	22	21	21	15	16	2	81	60
6 C.....	17	16	17	17	13	12	5	2	52	47
7 D.....	19	19	20	19	11	10	6	0	56	48
8 B.....	23	22	23	19	18	9	13	0	77	50
Total....	116	112	117	111	94	72	56	12	383	307
Per cent.....	.....	96.6	.....	94.9	.....	76.6	.....	21.4	.....	80.2

### SUMMARY

Seven wheat heads growing in the open were under practically continuous observation throughout their blooming periods, and nearly all of the 406 flowers on these heads were observed to bloom.

The time required for a wheat flower to open fully and the anthers to assume a pendent position varied considerably, but averaged 3 minutes 36 seconds for 25 flowers, while the time from beginning of opening to complete closing averaged 26.5 minutes.

Of all flowers under observation, 86.2 per cent bloomed during daylight, 6.9 per cent during twilight, and 6.9 per cent during the night. This study did not include the determination of the effect on blooming of the duration of the daily illumination period.

In most of the heads blooming began in the lower half of the uppermost third of the head. The lowest flower in the spikelet usually bloomed first and the others followed in order from lowest to highest, usually on successive days, although two flowers in the same spikelet sometimes bloomed on the same day.

Some of the upper flowers in certain spikelets bloomed before the lowest or lower flowers in other spikelets, depending on their position in the head.

Blooming on different heads extended over periods from about 101 to about 123 hours, a maximum of 25 flowers on one head blooming in a day.

Periods of intense and reduced blooming alternated throughout the day, the time of their occurrence depending to a considerable extent on temperature, rainfall, and sunshine. Blooming was observed at temperatures ranging from 56° to 78° F., inclusive.

The percentage of seeds set in the spikelet decreases from the lowest to the highest position, little difference existing, however, between the first and second positions.

In additional experiments flowers were observed to open without dehiscence of the anthers when submerged in water. Also, full and apparently normal blooming occurred in continuous darkness. Temperatures of 55° F. and below checked blooming in certain flowers, although pollen was discharged from anthers of flowers previously opened when the temperature was reduced as low as 52° F.

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# THE EFFECT OF SODIUM HYDROXID ON THE COMPOSITION, DIGESTIBILITY, AND FEEDING VALUE OF GRAIN HULLS AND OTHER FIBROUS MATERIAL<sup>1</sup>

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The aim of this investigation has been to improve the digestibility and feeding value of grain hulls and similar fibrous material. The agency used for this purpose has been sodium hydroxid, and its effect in varying concentrations on five different substances has been studied. The substances are oat hulls, barley hulls, rice hulls, cottonseed hulls, and flax shives.

It is of interest to note here that the annual output of oat hulls by three of the leading oat-milling concerns in the United States totals over 100,000 tons.<sup>3</sup> Although at the present time this by-product is mixed with the oat middlings and dust, which are also by-products of the mills, and is marketed as "oat feed," the product is admittedly of inferior feeding value, due to its high content of indigestible fiber. Any method the employment of which will bring about a considerable increase in digestibility of this and similar by-products is worthy of investigation. Aside altogether from their possible significance in a practical way, the facts brought out by the investigation are of considerable scientific interest.

Originality is not claimed for the method employed in the work. It was devised by Dr. Ernst Beckmann, of Berlin, Germany, for the purpose of hydrolyzing straw, and has been patented by him both in Germany and in the United States (3).<sup>4</sup> However, a careful search of the literature reveals that while considerable investigation has been carried on with straw, work with hulls has never before been attempted. The writer has studied the action of dilute sodium hydroxid as it affects the proximate and, to a certain extent, the ultimate composition of grain hulls, and has fed the untreated and treated hulls to sheep, ascertaining by the usual procedure of digestion experiments the effect of the alkali on the digestibility of the hulls.

## REVIEW OF THE LITERATURE

The review has been arranged under three headings: The chemistry of fibrous material; the action of sodium hydroxid and other alkalies on fiber; development of the process of hydrolyzing fibrous material for feeding purposes, together with the results of feeding experiments with the various products.

These different phases of the problem overlap more or less and some investigators have dealt with all three of them, but each forms a sufficiently clear-cut division to warrant dealing with them individually.

<sup>1</sup> Accepted for publication Nov. 24, 1923.

<sup>2</sup> This investigation was made under the direction of J. B. Lindsey. It was begun by C. L. Beals, who made a partial study of oat and rice hulls, and to whom due credit is given. It is published with the permission of the director of the Massachusetts Agricultural Experiment Station, and constitutes the review and experimental data of a thesis to be presented in partial fulfillment of the requirements for the degree of master of science in the graduate school of the Massachusetts Agricultural College.

<sup>3</sup> From approximate estimates furnished by the manufacturers.

<sup>4</sup> Reference is made by number (italic) to "Literature cited," p. 263-265.

## THE CHEMISTRY OF FIBROUS MATERIALS

The amount of work which has been done on the chemistry of plant fibers is nothing short of monumental, and yet, despite the vast accumulation of information now available, the chemistry of cellulose and of its combinations with pentosans, lignin, and allied substances in woody fiber is but imperfectly understood. Investigators are still at variance over the structure of the cellulose and the lignin molecule.

The writer has made no attempt to review all or even any considerable portion of the literature on the subject. A great deal of it is of a highly technical nature and of importance to the paper industry rather than to agriculture. It will be sufficient to outline the general concept of the chemistry of fiber as it exists at the present time, with such references as may be necessary.

Plant fibers are simply an aggregation of the cell walls of certain specialized cells occurring in the plant. These cell walls become elaborated, enlarged, and strengthened with age until maturity is reached, at which time, in high-fiber plants at least, they constitute the major portion of the individual cells and of the vegetative portion of the plant, the protoplasm having been almost, if not altogether, absorbed, or transported to the seed.

In the early stages of growth the cell wall is known to consist of practically pure cellulose, but with advancing age it becomes changed to a compound cellulose known as ligno-cellulose, which is characteristic of all fibrous or woody material and which imparts to such substances their property of rigidity. The process by which the cellulose is converted into ligno-cellulose is known as lignification. Just what this process involves in the way of chemical change and how it proceeds are still matters of dispute. Some investigators are of the opinion that it is purely a physical phenomenon; that the cellulose is simply embedded in, or incrustated by, the lignin; while others hold that the two are chemically combined and that the ligno-cellulose is formed at the expense of the cellulose.

Magnus (30, *p. 12*) considers that a definite linkage exists between the cellulose and the lignin.

Casparis (7) in a paper published in 1920 concludes that "lignified cell walls do not consist of chemically homogeneous material" and that "it appears likely that intramolecular formation of lignin takes place from the carbohydrates originally present in the cell wall."

Rassow and Zschenderlein (34) have evidence which points toward the pentosans as intermediate products in the formation of lignin.

Perhaps the most recent view of the process of lignification is that set forth by Esselen (12). He says in part:

It has been demonstrated that lignin is made up of hydrosols of high molecular weight which are absorbed from the sap by the cellulose fibers. . . . The maximum lignification coincides with the maximum percentage of absorbable colloidal substances in the sap. While the lignification depends mainly on the absorption referred to, it may be followed by certain chemical reactions, particularly dehydration, which manifest themselves in toughening and ageing.

In whatever way the transformation is brought about, the final product is the highly complex ligno-cellulose, the ultimate structure of which still baffles the chemist. It is, however, generally agreed that the complex consists of cellulose linked in some way with two noncellulose substances, one of which contains an aromatic nucleus, while the other, be-

cause it yields furfural on distillation with HCl, is presumed to be pentosan in nature. The latter two substances are so closely associated that they are grouped together under the term "lignin," a complex containing a considerably higher percentage of carbon than does cellulose (about 60 per cent) and less resistant to the action of alkali. For this substance many formulae have been proposed. For a fairly complete list of these the reader is referred to a recent article by Beckmann, Liesche, and Lehmann (4). The most recent empirical formula possessed of any degree of definiteness is that of F. Lehmann (4), who suggests  $C_{40}H_{44015}$ . The work of Melander (32) is more recent, but his results are not conclusive, several formulae being suggested.

Klason's conception that lignin is allied to coniferyl alcohol and derived from it by condensation and oxidation is worthy of consideration.

The outstanding characteristic of lignin, and one on which almost all authorities agree, is the presence in the molecule of methoxy ( $CH_3O$ ) and acetyl ( $CH_3CO$ ) groups. These are readily split off by the action of heat and dilute alkali with the formation of acetic acid, the residue left behind being much more stable and insoluble than the original lignin.

There are data to show that some of the methoxy groups are contained in the cellulose, but this is not definitely established, and the generally accepted idea is that the methoxy is characteristic of lignin. The subject is well summed up by Schorger (35) in a treatise on the chemistry of wood.

#### THE ACTION OF SODIUM HYDROXID AND OTHER ALKALIES ON FIBER

When such material as straw or wood is macerated with a solution of an alkali the solution becomes colored dark brown, and if the process is continued sufficiently the material is more or less completely disintegrated into a pulpy mass, which can be bleached by chlorin or any other suitable bleaching agent to a white or nearly white substance. This residue is a crude form of cellulose, which, depending upon its subsequent use, may or may not be further purified.

What are the changes involved in the destruction of the complex lignified tissue? What is it that the alkali removes? From this point on we shall consider only the action of dilute alkali on straw, as our problem is not the production of cellulose but simply the utilization of the principle in sufficient degree to render the fiber more digestible, at the same time holding at a minimum losses of valuable food substances.

The most clear and concise explanation is that given by Magnus in his "Theorie und Praxis der Strohaufschliessung" (30, p. 7-13). He considers that the reaction proceeds in a threefold manner:

Separation and solution of the silicic acid, which constitutes a portion of the incrusting substance of the straw and is present in most straws to the extent of 1 to 2 per cent, while some straws contain as much as 5 per cent.

Splitting off of the methoxy and acetyl groups from the lignin, of which they form a characteristic part. This results in the production of acetic acid with consequent neutralization of more or less of the alkali employed in the process. The lignin itself is also profoundly changed and is rendered more insoluble and inactive. It should be borne in mind, however, that complete, or nearly complete, solution and removal of the lignin can be brought about when desired by repeated treatment with alkali at higher temperatures than those successfully employed for straw hydrolysis. In paper manufacture this is what actually takes place.

Forcing or springing of the bonds which link the lignin and cellulose together. The theory of a linkage between these two substances in the fiber is advanced by Magnus, and he considers that the springing apart of these bonds is the most important

and essential feature in the action of the alkali. As a result, the intestinal bacteria of animals are enabled to attack the cellulose and split it up into simpler substances, such as sugars and organic acids which can then be utilized by the animal organism.

These changes take place at ordinary temperatures and the maximum action of the alkali is reached in a comparatively short time. It should be said, in addition, that coincident with these favorable changes there is more or less destruction of pentosans and cellulose by the alkali, but in the improved process patented by Beckmann (3) this unfavorable action is held at a minimum.

Neger (33) has advanced the idea that the mechanical effect of the alkali on the straw is also important, the middle lamella of the cell wall being dissolved and the thick-walled cells separated from one another.

The action of calcium hydroxid is similar to that of sodium hydroxid, but is less marked, the lignin and silicic acid being less attacked.

#### DEVELOPMENT OF THE PROCESS OF HYDROLYSIS OF FIBER

An endeavor has been made to cover the subject matter on this particular phase of the problem as completely as possible.

Practically all the work of developing a suitable process of fiber hydrolysis has been carried on in Germany, straw being the material generally used. Although many of the investigations were a result of the acute food shortage in that country during the war, the idea of utilizing processed fiber as an animal or even a human food is by no means a new one.

As early as 1865 Hellriegel and Lucanus (19) investigated the feeding value of straw which had been chopped up, moistened, and allowed to heat spontaneously. They concluded that such treatment diminished somewhat the food value of the straw.

In 1890 Henneberg and Lehmann (27) carried on feeding experiments with crude fiber prepared from rye straw by the action of sodium hydroxid. They concluded that cellulose prepared in this way was nearly equal in value as an albuminoid conservor to the easily soluble carbohydrates, and also that cellulose aided in fat production.

In 1894 Lehmann, as reported by Kellner (26, p. 288), showed that the food value of straw could be increased by cooking it with caustic soda in ordinary open kettles. In 1902 (28) he modified his process and made use of the pressure cookers of the paper industry, heating the straw and soda lye under pressure for several hours. The digestibility of straw thus treated was raised from 42 per cent to 56 to 60 per cent. However, the process has not come into general use.

In 1899 Kellner (26, p. 288) observed that rye straw hydrolyzed by the process used in paper manufacture had a digestibility of 88 per cent and was capable of producing more fat in ruminants than pure potato starch.

In 1906 Ustiantzev (44) found that cellulose from straw freed from incrusting substances had a decided food value and was equal to isodynamic quantities of starch and sugar as a protector of protein and fat. When fed to both rabbits and sheep it was almost completely digested.

Altmannsberger (1) in 1907 fed sheep with straw that had been treated with sodium hydroxid under pressure and found that the straw was readily eaten and that the digestibility of the crude fiber and ash had been materially increased.

About the same time, Diffloth (10) published data showing the increased value as a feeding stuff of straw from which the incrusting substance had been removed.

Grégoire (17) reported in 1907 on the method of Seidl and Bauriedl in which straw was treated under pressure with 3 per cent NaOH. The material was fed while still wet and was claimed to be fully as digestible as starch.

After the outbreak of the World War, investigations of this nature became quite numerous. One of the first processes proposed was that of Oexmann, who utilized the straw pulp of the paper industry, mixing it with 35 per cent of molasses. The mixture was dried, ground, and placed on the market as "Strohkraftfutter II." With protein added to it, it was known as "Strohkraftfutter I."

Lehmann's process of cooking the straw under pressure with varying concentrations of NaOH was also further experimented with at this time, but was found to be not economically sound, due to costly equipment and the necessity of handling large amounts of water.

Colsmann's process was devised to overcome these defects and consisted of cooking straw without pressure for 12 hours, using simple equipment. The element of time was the main consideration here, and in that respect the Muller process, which shortened the time somewhat by stirring, was an improvement over Colsmann's method.

The Dahlemer process is similar in principle to Colsmann's and to Lehmann's original method, cast-iron vessels being employed for the hydrolysis of the straw.

Fingerling reports that Colsmann's product had a digestibility of 60 to 65 per cent (26, p. 290), while that of the Dahlemer process was 75 per cent digestible (13, p. 6).

Unfortunately, we have not been able to find in the literature any original accounts of the Oexmann, Colsmann, Muller, and Dahlemer processes, but they are reported in some detail by Fingerling (26, p. 289, 290) and by Magnus (30, p. 1-4). Because of the lack of references we are unable to assign definite dates as to the chronological sequence of their publication or introduction into practice. It is inferred, however, from a careful study of the literature that they were all developed during the early part of the war. Other investigators about the same time were Stutzer (42), Dannfelt (9), Tollens (43), and Hansen (18).

All of the processes devised for straw hydrolysis up to as late as 1917 required as an essential feature of their operation the application of heat. In 1918 Beckmann put forward his process of hydrolysis in the cold, which was so much more simple and economical that it rapidly superseded those already in use and became the subject of careful investigation by the German experiment stations and others. Since that time practically all the literature on the subject (in Germany at least) deals with this process or modifications of it. The process has been patented, in Germany about 1919, and in the United States more recently (3).

The essential features of the method are hydrolysis of the material with eight times its weight of 1.5 per cent NaOH in open vats for a comparatively short time—three hours is usually sufficient—draining off the liquor and washing with water until the product no longer turns red litmus paper blue. The process is carried on at ordinary temperature, and those who have thoroughly investigated the method claim that the hydrolysis is as complete at this temperature and in the relatively short time recommended as it is when the material is subjected to cooking either with or without pressure for longer periods of time. Also, the loss of valuable nutritive substance is very much reduced.

The method is discussed in considerable detail by Magnus (30) and also by Fingerling (26, p. 291, 292).

Magnus has reviewed and discussed in his text on the subject (30) all of the more important processes of straw hydrolysis which had been devised up to the date of its publication in 1919. In addition, he has given a detailed account of the theory of straw hydrolysis and of many of his own investigations. Though rather out of date now, it is the only text on the subject of which the writer is aware.

In 1919 Jonscher (25) reported on his investigations in treating straw meal and wood meal with HCl and (or) NaOH, recommending some of the products as suitable for animal and human food.

Within the past four years Honcamp and his coworkers have carried on quite extensive investigations into the relative merits of various methods of straw hydrolysis. In his first paper (21), published in 1919, he reports unfavorably on the methods proposed by Minck and Schwalbe for hydrolysis with hydrochloric acid. Such treatment makes the straw no more digestible.

His second paper (22, p. 1-41), published in 1921, deals with hydrolysis of straw by calcium hydroxid without pressure. Such treatment increases the starch value above that of the original straw to about the same extent as does sodium hydroxid. Loss of organic substance is greater when the hydrolysis takes place under pressure than by simply boiling.

In his third contribution (22, p. 42-63), published coincident with the second, he discusses the effect of hydrolyzing with sodium carbonate, which is similar to that of NaOH and  $\text{Ca}(\text{OH})_2$ . Using concentrations of  $\text{Na}_2\text{CO}_3$  similar to those of NaOH and  $\text{Ca}(\text{OH})_2$  employed, the fodder value of the straw was considerably improved.

His fourth paper (23), published about the same time as the second and third, deals with hydrolysis by sodium hydroxid under pressure. Only cereal straw is suitable for such treatment. Results obtained with a definite amount of NaOH (3.5 kg. per 100 kg. of straw) were about the same as where twice that amount was used.

In his most recent work (24) Honcamp investigated the Beckmann methods, using both sodium hydroxid and calcium hydroxid. His conclusion was that the loss in crude and digestible nutrients was greater with NaOH than with  $\text{Ca}(\text{OH})_2$ .

In addition to this series of five papers, he published a general paper (20) in 1919 with some rather important conclusions. He states that pressure cooking results in greater destruction of organic matter than when the cooking is done in open vessels, and that hydrolysis with NaOH results in a substantial increase in digestibility in rye, barley, and oat straw, but only slight increase in pea, seed beet, and rape straw.

Semmler and Pringsheim (40) found that usually less than 50 per cent of the crude fiber of natural products is digested when the lignin content is in excess of 20 per cent, but up to 75 per cent may be digested in the case of straw hydrolyzed by sodium hydroxid, despite a much higher lignin content.

Fingerling has made some important contributions to the subject. In addition to his numerous experiments already referred to (13, and 26, p. 289-292), he has investigated Beckmann's process quite thoroughly. In 1919 he published a paper (14) dealing with the influence of time of hydrolysis upon the amount of nutritive material liberated. The results showed that the greatest amount of hydrolysis took place in the first three hours, and that action of the NaOH was practically completed

in four hours. In a second paper (15), published in 1922, he shows quite conclusively that, within reasonable limits, the stronger the NaOH solution used the higher is the digestibility of the hydrolyzed straw.

Wagner and Schöler (46) treated straw with 2 per cent lye by the Beckmann's process and found that the product when fed to sheep was very serviceable fodder, fed either wet or dry.

The work of Scurti et al., reported in 1919 and later, is worthy of mention. They have investigated the influence of hydrolysis on the composition and nutritive value of corn cobs (36), wheat straw (37), grapevine shoots, and hemp (38). While sulphuric acid was the principal hydrolyzing agent used, nitric and hydrochloric acids and sodium hydroxide were also employed. The products from wheat straw and corn cobs were compressed into cakes and fed to farm animals with fair success (39).

In 1919 Ellenberger (11) reported some experiments with hydrolyzed wood meal as a feed for working horses. He concludes that this material may not only be substituted for the hay of the ration but may also replace the oats if some supplemental protein is furnished.

Völtz (45) in 1920 treated straw and chaff by Beckmann's process, and reports considerably more digestible nutrients in chaff treated for 18 hours than in chaff treated for 3 hours. Straw treated for 24 hours contained slightly more digestible nutrients than straw treated for 12 or 72 hours.

In 1920 Godden (16) published an account of his method of straw hydrolysis, which he devised for small-scale operations and which differs somewhat from any of the German processes. The chopped straw is soaked overnight in 1.5 per cent of NaOH and then steamed for an hour in a specially constructed boiler. After draining and cooling it is fed immediately. The dry matter of the treated straw has approximately 1.5 times the value of the original dry matter, and for production purposes its value is nearly doubled. He concludes that the gain in nutritive efficiency compensates for the loss in dry matter, but emphasizes the need of further investigation of the possibilities of such treatment.

Weiser and Zaitschek (47) carried on an investigation similar to one by Fingerling (15) in which they studied the effect of the amount of soda used on the digestibility of straw. The Lehmann apparatus was used and, contrary to the findings of Fingerling, they found that the highest starch values were obtained when the NaOH solution used was weakest. From the large number of variables in their experiments we are inclined to view their results with some misgivings.

Sherrard and Blanco (41) have described a method for preparation of a cattle food from hydrolyzed sawdust. The product was fed to three cows at the Wisconsin College of Agriculture "with highly gratifying results." The essential feature of the method consisted in the digestion of the sawdust with 1.8 per cent sulphuric acid under pressure. About 21 per cent of the original wood meal was converted into sugar.

Braunschild (6) patented in 1921 a process for treatment of substances rich in cellulose with a strong solution of calcium chloride.

Blasweiler (5) has recently described Steffen's method of straw digestion. Straw is cooked under pressure for one and one-half hours with 10 per cent sodium hydroxide. The product has a composition similar to that obtained by Oexmann's process.

From the many investigations cited, it is clear that the action of various hydrolyzing agents upon straw, while attended with some loss, noticeably improves its digestibility. Sodium hydrate proved to be the most effective agent, followed closely by calcium hydrate, the latter naturally proving the more economical.



## EXPERIMENTAL

As already stated, the method of hydrolysis employed for the treatment of grain hulls in this investigation was that of Beckmann. The apparatus consisted of a tank constructed of 2-inch spruce planking, coated on the inside with asphalt, and provided with a strainer and outlet tap at the bottom to drain off the lye and wash water. The inside dimensions of this tank are: Length, 6 feet; breadth, 3 feet; depth, 1 foot 6 inches. Fifteen kilos of grain hulls can be readily handled in it at one time. In addition to the tank, a homemade filter press, for removal of the excess water after hydrolysis, and an eight-compartment special drying oven constituted the major portion of the equipment.

The procedure in treatment of the hulls was as follows: An amount of sodium hydroxid equivalent in weight to eight times the amount of hulls used was made ready in the tank, the exact strength being adjusted by titration and addition of more NaOH or water as required. The desired amount of hulls was weighed out and transferred at once to the tank, where it was thoroughly mixed with the alkali by means of a wooden hoe. The strengths of sodium hydroxid used were 1, 1.5, and 3 per cent. The 1.5 per cent strength is that employed by Beckmann. The 3 per cent strength, used with rice hulls only, was employed in order to ascertain whether it would have any more marked action on the very woody, gritty rice hulls than did the 1.5 per cent strength. The 1 per cent strength was used with the idea of economy in mind. The mixture was allowed to stand for three hours, with frequent stirring; the soda liquor was then drained off as completely as possible and the hulls thoroughly washed with cold water until the wash water no longer showed a pink tinge with phenolphthalein, about six changes of water being usually sufficient. The hulls were then transferred to the filter press, where the excess water was removed, and they were finally spread out in as thin layers as possible in shallow galvanized pans and dried in the special steam oven. When dry they were bagged and stored until such time as the digestion experiments could be carried on, which, as a matter of fact, was almost immediately.

As in all the digestion work done at this station, sheep were used, all the individuals employed being aged wethers well trained in the routine of the work. This is a detail of no small significance, as anyone can attest who has attempted work of this kind. The feeding trials were carried on in the usual manner, ample details of which are given in an earlier publication of this station (29). Two sheep were used throughout each trial. The hulls, both treated and untreated, were fed at the rate of 100 gm. <sup>5</sup> daily, along with a basal ration <sup>6</sup> of 500 gm. English hay, 150 gm. gluten feed, 10 gm. salt, and water ad libitum.

The hulls were mixed with the gluten feed, and no trouble was experienced in getting the sheep to eat them. No further preparation of the hulls was necessary except in the case of the rice hulls, which had to be ground before the sheep would eat them.

Feeds and feces were submitted to the regular fodder analyses, according to the methods of the Association of Official Agricultural Chemists,<sup>7</sup>

<sup>5</sup> In the case of the untreated oat hulls and rice hulls, and the oat hulls and rice hulls treated with 1.5 per cent sodium hydroxid, the amount of hulls fed was 150 gm.

<sup>6</sup> The basal ration for the trials in which untreated oat hulls and oat hulls treated with 1.5 per cent sodium hydroxid were fed contained no gluten feed; otherwise it was identical with that given above.

<sup>7</sup> Association of Official Agricultural Chemists. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS, AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. Revised to Nov. 1, 1919. p. 71, 72, 97-98. Washington, D. C. 1920.



and in addition the percentage of starch was determined in the hulls, and pentosans and lignin in all the feeds and solid excreta. Pentosans were determined according to the official method as described in the manual of the Association of Official Agricultural Chemists.<sup>8</sup>

Starch was determined by pancreatin in the following manner:

Two grams of finely ground (100-mesh) material were transferred to a hardened filter paper and washed with several portions of hot 10 per cent ethyl alcohol to remove the sugars. The residue was immediately transferred by means of a stemless funnel and a minimum of water from a wash bottle to a 250 cc. volumetric flask. The suspension, if not already diluted sufficiently by the water used for transferring, was further diluted to about 100 cc. and boiled for half an hour to rupture the cell walls and the starch granules and liberate the starch. The flask was then filled almost to the mark with distilled water and allowed to cool to below 37° C. A pinch of sodium bicarbonate was then added to insure slight alkalinity for optimum action of the pancreatin, followed by one-tenth of a gram of full-strength pancreatin.<sup>9</sup> The solution was at once made up to the mark, shaken well, and placed in a water bath at 37° to 40° C. for half an hour, at the end of which time the contents of the flask were emptied into a 500 cc. beaker to facilitate subsequent pipetting; 200 cc. of the liquid were immediately pipetted off into another 500 cc. beaker as rapidly as possible, and 20 cc. of HCl (sp. gr. 1.125) added at once, thus inactivating the pancreatin. The 220 cc. of liquid were then filtered by suction into another 250 cc. flask, using a platinum cone to support the filter paper, and a bell jar of suitable size on a ground glass plate to hold the flask into which the filtrate passed. As soon as possible after filtration was complete, the flask was placed under a reflux condenser and heated gently for two hours, the solution cooled, nearly neutralized with NaOH, and made up to 250 cc. With some materials another filtration was necessary at this point, but suction was not required. Reducing sugar was determined in aliquots of the solution by Allihn's modification of Fehling's method.<sup>10</sup>

Lignin was determined by a modification of the method of Ost and Wilkening (reported by Cross and Bevan, 8, p. 39), which was proposed by Mahood and Cable (37). The principle of the method consists in the hydrolysis of all the constituents of the material except the lignin by means of concentrated sulphuric acid. The dissolved substances are removed by filtration and washing and the residue is dried and weighed as lignin. The details are as follows:

Two <sup>11</sup> grams of the material were extracted with ether in an ordinary fat extraction apparatus, transferred to a 1,000 cc. Erlenmeyer flask and covered with 10 times its weight (13 cc.) of 72 per cent sulphuric acid. Considerable care and some practice were necessary at this point in order to get all particles of the dry material in contact with the relatively small amount of acid. The hydrolysis was allowed to proceed for 16 hours at room temperature, at the end of which time the solution was diluted with ordinary tap water to a strength of 3 per cent (480 cc. of water was the amount necessary for that degree of dilution). The solution was then boiled under a reflux condenser for 2 hours, filtered through linen, washed with hot water, transferred to a tared Gooch crucible, dried at 100° C., weighed, ignited, and weighed again. The loss in weight was considered as lignin.

Some preliminary work was done in determining lignin by the method of Willstätter (reported by Magnus, 30, p. 14), in which 42 per cent hydrochloric acid is used as the hydrolyzing agent, instead of sulphuric acid. This method proved less satisfactory because of the cost of HCl so highly concentrated, the difficulty of keeping it at that strength, and the extreme unpleasantness of the dense fumes given off.

<sup>8</sup> OP. CIT., p. 96.

<sup>9</sup> This material was furnished to us through the courtesy of Parke, Davis & Co., of Detroit, Mich., and, unlike the ordinary reagent, contained no diluent.

<sup>10</sup> Association of Official Agricultural Chemists. OP. CIT., p. 90.

<sup>11</sup> Mahood and Cable (37) use 4 gm., but half that quantity was better adapted to our purposes.

## PRESENTATION AND DISCUSSION OF EXPERIMENTAL DATA

The results secured in the investigation group themselves under two heads: Effect of the sodium hydroxid on the composition of the hulls; effect on the digestibility and feeding value of the hulls.

## EFFECT OF SODIUM HYDROXID ON THE COMPOSITION OF THE HULLS

This group of results can be subdivided into four distinct topics, which follow:

## LOSSES IN WEIGHT IN THE TREATED MATERIALS DUE TO THE ACTION OF THE SODIUM HYDROXID, AND REDUCTION IN THE STRENGTH OF THE SODIUM HYDROXID

The loss in weight was ascertained on a dry-matter basis by weighing the material and making dry-matter determinations, both before and after treatment. The reduction in strength of the soda was determined by titration with N/2 sulphuric acid. The following table sets forth the figures:

TABLE I.—*Loss in weight on treatment with soda and reduction in strength of soda.*

Material.	Loss in weight on treatment (dry-matter basis).	Strength of NaOH employed.	Strength of NaOH after use.	NaOH consumed.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Oat hulls.....	16.54	1.5	0.70	53.33
Do.....	10.74	1	.46	54.00
Barley hulls.....	20.29	1.5	1.04	30.66
Do.....	19.31	1	.64	36.00
Rice hulls.....	15.60	1.5	.75	50.00
Do.....	19.51	3	2.28	24.00
Cottonseed hulls.....	9.69	1.5	.75	50.00
Flax shives.....	25.03	1.5	.80	46.66

These losses are due, as already explained, to solution and separation of the silicic acid, a portion of the lignin, more or less of the cellulose and pentosans, and to unavoidable mechanical loss. In draining off the soda liquor and subsequent wash water, more or less of the fine particles of the treated hulls passed through the finest sieve that it was practicable to use. This was especially true of the flax shives, as they were ground quite fine when received. The mechanical loss, however, is believed to have been small.

The average loss in weight for the several substances when 1.5 per cent NaOH was used was equivalent to 17.43 per cent of the original material; when 1 per cent NaOH was used the average loss was slightly less—15.03 per cent; and in the single instance where 3 per cent NaOH was employed the loss was slightly higher—19.5 per cent; indicating that the stronger the soda solution the greater the loss, and that for a given strength of soda the loss depended upon the character of the material treated.

The reduction in strength of the sodium hydroxid is due to neutralization by the acetic acid formed. In this connection it may be worth while to record our observation of what took place when the hulls were added to the NaOH solution in the tank. During the first

few minutes of the treatment there was always a noticeable formation of small bubbles, accompanied by a quite audible crackling sound suggestive of a mild effervescence. This phenomenon ceased to be perceptible after the first 15 or 20 minutes, which would seem to indicate a slowing up of the reaction and that the action of the alkali takes place largely in the first few minutes. These assumptions are borne out by the work of Magnus (30, p. 12) and of Beckmann (3).

The average amount of alkali consumed or neutralized in the process was 45 per cent of the total amount when the strength employed was 1 per cent; 46.66 per cent when the strength employed was 1.5 per cent; 24 per cent when the strength employed was 3 per cent. In other words, the percentage strength of the exhausted solutions averaged 0.45 per cent, 0.70 per cent, and 2.28 per cent, respectively, for the 1 per cent, 1.5 per cent, and 3 per cent NaOH solutions.<sup>12</sup>

It will be noted that the percentage amount of NaOH consumed is about the same for the 1 per cent and 1.5 per cent—around 45 per cent, or slightly less than half of the total amount employed—while the actual amount consumed is about the same for the 1.5 per cent and 3 per cent solutions, namely, about 0.7 per cent.

Beckmann recommends the use of the exhausted alkali a second and even a third time, bringing it back to the desired strength by adding the required amount of fresh sodium hydroxid. In the practical operation of the process this procedure is in the interest of economy, but for our experimental work we considered it inadvisable, and accordingly a fresh solution was prepared for each lot of hulls.

#### REACTION OF THE MATERIALS BEFORE AND AFTER TREATMENT

In order to ascertain if there was any residual uncombined alkali in the treated materials, the water-soluble acidity or alkalinity of all the treated and untreated samples was determined according to the method given in the manual of the Association of Official Agricultural Chemists.<sup>13</sup> Table II sets forth the results.

TABLE II.—*Water-soluble acidity or alkalinity of untreated and treated hulls*

Laboratory No.	Material.	N/10 NaOH required per gram of substance.	N/10 H <sub>2</sub> SO <sub>4</sub> required per gram of substance.
		<i>Cc.</i>	<i>Cc.</i>
248	Oat hulls, untreated.....	0.23	.....
503	Oat hulls, treated 1 per cent NaOH.....	.10	.....
293	Oat hulls, treated 1.5 per cent NaOH.....	.....	0.16
453	Barley hulls, untreated.....	1.14	.....
491	Barley hulls, treated 1 per cent NaOH.....	.23	.....
482	Barley hulls, treated 1.5 per cent NaOH.....	.42	.....
337	Rice hulls, untreated.....	.22	.....
321	Rice hulls, treated 1.5 per cent NaOH.....	.....	.07
512	Rice hulls, treated 3 per cent NaOH.....	.....	.08
449	Cottonseed hulls, untreated.....	.23	.....
460	Cottonseed hulls, treated 1.5 per cent NaOH.....	.08	.....
485	Flax shives, untreated.....	.47	.....
494	Flax shives, treated 1.5 per cent NaOH.....	.08	.....

<sup>12</sup> The 3 per cent NaOH solution was used in only one instance, hence the corresponding value does not represent an average.

<sup>13</sup> Association of Official Agricultural Chemists. *OP. CIT.*, p. 98.

It is seen that all untreated materials showed a slight water-soluble acidity measured in terms of N/10 NaOH, varying from 0.22 cc. N/10 NaOH in the case of rice hulls to 1.14 cc. in the case of barley hulls. A slight acidity is normal to most feeding stuffs. The relatively high acidity of the barley hulls may be attributed to a slight fermentation of the considerable amount of carbohydrate present which had not been separated from the hulls. In the case of the treated materials, five lots showed a slight acidity, varying from 0.08 cc. to 0.42 cc. N/10 NaOH, and three lots showed a slight alkalinity, from 0.07 cc. to 0.16 cc. N/10 H<sub>2</sub>SO<sub>4</sub> per gram of substance. From these results it is evident that in the majority of cases there was no residual uncombined soda from the treatment left in the materials after thorough washing, and in those cases where there was any alkalinity it was so slight that it would be readily taken care of by the HCl in the animal's stomach. Care should, however, be used to wash out the soda after treatment as thoroughly as possible compatible with good practice.

#### EFFECT OF THE TREATMENT ON THE PROXIMATE CONSTITUENTS OF THE HULLS

The detailed results of all the proximate analyses, together with percentage increases or decreases of the various constituents due to the action of the alkali, are given in the accompanying table.

TABLE III.—Summary of the proximate analysis of grain hulls (untreated and treated)

Laboratory No.	Material.	Moisture as fed.	Dry matter basis.				
			Total ash.	Crude protein.	Crude fiber.	N-free extract.	Crude fat.
248	Oat hulls, untreated.....	7.70	6.33	2.26	33.24	57.24	0.93
503	Oat hulls, treated 1 per cent NaOH.....	5.77	5.69	2.05	34.80	56.99	.47
	Percentage increase or decrease due to treatment.....		-10.12	-9.30	+4.69	-1.44	-49.47
293	Oat hulls, treated 1.5 per cent NaOH.....	4.05	5.23	1.37	40.20	52.65	.55
	Percentage increase or decrease due to treatment.....		-17.38	-39.39	+20.93	-8.02	-40.86
337	Rice hulls, untreated.....	6.25	19.06	3.02	47.80	35.38	.75
321	Rice hulls, treated 1.5 per cent NaOH.....	3.61	17.46	2.51	46.08	33.41	.58
	Percentage increase or decrease due to treatment.....		-8.40	-16.89	+10.23	-5.57	-22.67
512	Rice hulls, treated 3 per cent NaOH.....	5.92	13.13	1.83	50.26	34.23	.55
	Percentage increase or decrease due to treatment.....		-31.12	-39.40	+20.23	-3.25	-26.67
453	Barley hulls, untreated.....	6.76	4.50	10.81	15.56	66.86	2.27
491	Barley hulls, treated 1 per cent NaOH.....	6.24	4.74	10.41	18.97	63.89	1.99
	Percentage increase or decrease due to treatment.....		+5.33	-3.63	+21.91	-4.45	-12.34
482	Barley hulls, treated 1.5 per cent NaOH.....	5.09	4.33	9.25	19.03	65.40	1.99
	Percentage increase or decrease due to treatment.....		-3.78	-14.44	+22.30	-2.18	-12.34
449	Cottonseed hulls, untreated.....	6.93	2.13	4.08	43.99	48.60	1.19
460	Cottonseed hulls, treated 1.5 per cent NaOH.....	5.15	2.75	3.03	49.83	43.55	.84
	Percentage increase or decrease due to treatment.....		+29.10	-25.74	+13.27	-10.40	-29.42
485	Flax shives, untreated.....	6.78	4.09	5.24	53.81	35.05	1.81
494	Flax shives, treated 1.5 per cent NaOH.....	5.54	5.35	4.41	62.84	26.07	1.33
	Percentage increase or decrease due to treatment.....		+30.81	-15.84	+16.77	-25.63	-26.52

+ Increase.

- Decrease.

The treatment relatively increased the fiber and decreased all other constituents except ash, which was increased in three instances. The considerable increase in ash in cottonseed hulls and flax shives can probably be explained by considering it as a relative increase, the ash of these

materials being so insoluble that it was unattacked by the alkali, while a portion of the more soluble organic constituents was removed. It is possible also that sufficient residual soda remained combined in these materials to account for the ash increase.

The striking feature of the results is the appreciable percentage increase of fiber in all cases, due to the removal of the more soluble portions of the hulls. One notices also that the increase in fiber was consistently greater the higher the concentration of sodium hydroxid. Although the protein and fat suffered considerable loss, they are present in relatively small percentages and hence are of minor importance. (See Table IV.)

TABLE IV.—Net loss in pounds on a dry-matter basis of each proximate constituent

Material.	Loss in pounds for each 100 pounds of dry matter treated.					
	Total ash.	Crude protein.	Crude fiber.	N-free extract.	Crude fat.	Total.
Oat hulls, treated 1 per cent NaOH.....	1.26	0.43	2.18	6.37	0.51	10.75
Oat hulls, treated 1.5 per cent NaOH.....	1.97	1.12	a .31	13.30	.47	16.55
Barley hulls, treated 1 per cent NaOH.....	.68	2.41	.25	15.51	.66	19.51
Barley hulls, treated 1.5 per cent NaOH.....	1.05	3.44	.39	14.73	.68	20.29
Rice hulls, treated 1.5 per cent NaOH.....	4.32	.90	2.91	7.18	.26	15.57
Rice hulls, treated 3 per cent NaOH.....	8.49	1.55	1.35	7.83	.31	19.53
Cottonseed hulls, treated 1.5 per cent NaOH.....	a .35	1.34	a 1.01	9.27	.43	9.68
Flax shives, treated 1.5 per cent NaOH.....	.08	1.93	6.70	15.51	.81	25.03

a These figures represent gain instead of loss. In the case of the crude fiber an absolute gain is impossible, so these small increases are explainable only on the basis of analytical or experimental error. In the case of the ash which showed an absolute increase it is possible that residual NaOH might account for it.

Considering the results from this angle, we see that the fiber was practically unattacked in so far as its removal by solution was concerned, while the greater or lesser amounts of all the other constituents were removed. The greatest actual losses were in the case of the nitrogen-free extract, which includes the starch, a portion of the pentosans and lignin, and allied substances.

#### EFFECT OF THE TREATMENT ON SOME OF THE ULTIMATE CONSTITUENTS OF THE HULLS

It was thought that a more detailed analytical examination of the hulls than that involved in the conventional fodder analyses would furnish still more accurate information as to the chemistry involved in the process. Table V gives the results of starch, pentosan, and lignin determinations made as described in a previous section of this paper.

The figures in Table V show that relatively the starch was increased in all instances, the pentosans in all but one instance, and the lignin in five out of eight. In a general way, the increases in pentosans and lignin parallel those for fiber, which is what would be expected.

One is impressed with the high percentage of pentosans in the untreated oat hulls; in fact, pentosans together with the lignin make up the major portion of the hulls. The rice hulls are composed largely of ash, fiber, including pentosans, and lignin. They are somewhat more lignified than the oat hulls, and the presence of the lignin together with the high ash percentage accounts for their inferior nutritive value. Cottonseed hulls with their very high crude fiber percentage, together with the large amount of pentosans and lignin, are in the same class with the rice hulls. Flax shives, containing approximately 54 per cent

of fiber, 27 per cent of pentosans, and 33 per cent of lignin, the most lignified of the several substances examined, should prove to be the least digestible.

TABLE V.—*Starch, pentosans, and lignin in grain hulls (untreated and treated)*

Material (dry matter basis).	Starch per cent.	Pentosans per cent.	Lignin per cent.
Oat hulls, untreated . . . . .	4.73	40.02	20.20
Oat hulls, treated 1 per cent NaOH . . . . .	5.59	43.80	23.54
Percentage increase or decrease due to treatment	+18.18	+9.45	+16.53
Oat hulls, treated 1.5 per cent NaOH . . . . .	7.67	44.16	18.61
Percentage increase or decrease due to treatment	+62.16	+10.34	-7.87
Rice hulls, untreated . . . . .	5.65	21.98	22.72
Rice hulls, treated 1.5 per cent NaOH . . . . .	5.67	24.10	23.09
Percentage increase or decrease due to treatment	+0.35	+9.65	+1.63
Rice hulls, treated 3 per cent NaOH . . . . .	5.67	25.28	25.10
Percentage increase or decrease due to treatment	+0.35	+15.01	+10.48
Barley hulls, untreated . . . . .	13.41	23.50	14.87
Barley hulls, treated 1 per cent NaOH . . . . .	15.78	26.61	15.83
Percentage increase or decrease due to treatment	+17.67	+13.23	+6.46
Barley hulls, treated 1.5 per cent NaOH . . . . .	22.55	26.21	15.01
Percentage increase or decrease due to treatment	+68.16	+11.53	+0.94
Cottonseed hulls, untreated . . . . .	5.87	32.52	25.29
Cottonseed hulls, treated 1.5 per cent NaOH . . . . .	6.08	34.10	21.97
Percentage increase or decrease due to treatment	+3.58	+4.86	-13.13
Flax shives, untreated . . . . .	6.26	27.16	33.28
Flax shives, treated 1.5 per cent NaOH . . . . .	6.89	25.92	32.40
Percentage increase or decrease due to treatment	+10.06	-4.57	-2.64

+ Increase.

- Decrease.

The losses due to treatment seem to be distributed between the ash, protein, fat, pentosans, and lignin. The most pronounced losses occur in the pentosans and lignin. Even after all of these are accounted for, there is more or less loss of alkali-soluble constituents not identified, which because of a lack of complete identification are still grouped under the term nitrogen-free extract. In fact, in substances of this nature the nonnitrogenous materials are of such a complex nature and are so interwoven with each other that it does not appear possible to determine the different constituents with quantitative exactness. This difficulty in the case of starch has led Armsby (2, p. 72) to remark that "unfortunately starch can be determined only more or less approximately."

TABLE VI.—*Net loss of starch, etc., due to treatment*

Material.	Loss in pounds for each 100 pounds of dry matter treated.		
	Starch.	Pento- sans.	Lignin.
Oat hulls, treated 1 per cent NaOH . . . . .	0.26	0.98	0.81
Oat hulls, treated 1.5 per cent NaOH . . . . .	1.67	3.16	4.67
Barley hulls, treated 1 per cent NaOH . . . . .	.68	2.03	2.10
Barley hulls, treated 1.5 per cent NaOH . . . . .	4.56	2.61	2.91
Rice hulls, treated 1.5 per cent NaOH . . . . .	.86	1.64	3.23
Rice hulls, treated 3 per cent NaOH . . . . .	.91	1.63	2.52
Cottonseed hulls, treated 1.5 per cent NaOH . . . . .	.38	1.72	5.45
Flax shives, treated 1.5 per cent NaOH . . . . .	1.09	7.73	8.99

\* Represents a gain, which of course is impossible and is explainable only on the basis of analytical or experimental error.

## EFFECT OF SODIUM HYDROXID ON THE DIGESTIBILITY AND FEEDING VALUE OF THE HULLS

This is revealed by a study of the digestion coefficients of the various constituents of the hulls. For the purposes of this investigation we need consider only the digestion coefficients of the total dry matter, crude fiber, nitrogen-free extract, pentosans, and lignin. These are the important constituents, the protein, fat, and ash being present for the most part in unimportant amounts. Table VII presents the results in condensed form. For the benefit of the critical student it is appropriate to remark that the digestion trials were conducted with the greatest care. Where the coefficients from two individuals differ materially, it should be borne in mind that work of this nature can not be controlled in the same way that laboratory determinations can be. Biological processes are too complex to permit it; and it frequently happens also that the individuality of the animal exerts an influence on the final result. Accordingly the coefficients must be viewed as giving general rather than absolutely definite results.

Careful scrutiny of the individual and average digestion coefficients reveals the following facts:

The digestibility of the total dry matter was substantially increased by treatment in case of the oat, barley, and rice hulls.

The digestibility of the crude fiber, nitrogen-free extract, and pentosans in oat, barley, and rice hulls was markedly increased by treatment.

The results in case of lignin are not so consistent, but it must be remembered that treatment has changed its molecular structure, hence the data can not be considered of any particular value. It seems evident that in the untreated material, in several instances, some little use was made of the lignin complex, the results varying with the different materials. After treatment, in case of the oat, rice, and barley hulls the digestibility of the lignin residue seems to have been somewhat improved. In view, however, of our incomplete knowledge of the structure of the lignin molecule and of the varying results secured with two sheep on the same material, it may be concluded that lignin is of quite doubtful value as a source of nutrition. Some investigators consider it to be entirely indigestible.

In addition to the facts mentioned under Table VII, the following general statements seem to be warranted:

Expressed on a percentage basis, treatment with varying strengths of dilute NaOH invariably increased the total digestible dry matter of oat hulls, barley hulls and rice hulls. In the majority of cases it also increased markedly the digestibility of the important constituents of these materials.

Cottonseed hulls and flax shives appear to have been unaffected by the treatment. It is probable that the lignin-cellulose linkage was broken only to a slight extent, due no doubt to the higher degree of lignification in these materials, as compared with the hulls of the cereal grains.

Although the rice hulls showed by far the greatest response to the action of the soda, the original material was so much inferior in digestibility to the untreated oat and barley hulls that the net result of treatment was a product considerably inferior in total digestible nutrients even to the untreated oat hulls; hence the action of soda on this material is not likely to be of economic value.

TABLE VII.—*Digestion coefficients (individual results and average of two sheep)*

Material	Sheep.	Total dry matter.	Crude fiber.	N-free extract	Pentosans.	Lignin.
Oat hulls, untreated	15	36.37	56.32	31.73	33.43	11.23
Do.	11	36.11	48.82	36.42	38.38	(a)
Average		36.24	52.57	34.08	35.91	
Oat hulls, treated 1 per cent NaOH	16	26.50	57.88	26.65	51.18	(a)
Do.	17	70.37	82.77	64.67	71.07	39.63
Oat hulls, treated 1.5 per cent NaOH	12	73.31	84.91	71.55	84.50	28.15
Do.	13	87.90	97.22	86.53	62.99	46.56
Average		80.61	91.07	79.04	73.78	37.36
Increase due to treatment (1 per cent NaOH)		b 34.13	b 30.20	b 30.59	b 35.16	b 28.40
Percentage increase		b 94.17	b 57.43	b 89.76	b 97.91	b 252.89
Increase due to treatment (1.5 per cent NaOH)		44.37	38.50	44.96	37.87	26.13
Percentage increase		122.43	73.24	131.92	105.46	232.68
Barley hulls, untreated	16	65.77	42.73	64.24	50.02	18.76
Do.	17	53.17	46.86	55.47	44.77	(a)
Average		59.97	44.79	59.85	47.40	
Barley hulls, treated 1 per cent NaOH	16	64.56	37.99	70.04	52.87	23.85
Do.	17	72.83	66.55	79.57	76.91	45.55
Average		68.69	52.28	74.81	64.89	34.70
Barley hulls, treated 1.5 per cent NaOH	16	79.49	70.76	81.98	73.91	(a)
Do.	17	85.52	91.80	87.22	92.16	(a)
Average		82.50	81.28	84.60	83.04	
Increase due to treatment (1 per cent NaOH)		8.72	7.49	14.96	17.49	15.96
Percentage increase		14.54	16.72	25.00	36.90	84.97
Increase due to treatment (1.5 per cent NaOH)		22.53	36.49	24.75	25.64	(a)
Percentage increase		37.57	81.47	41.35	75.19	(a)
Rice hulls, untreated	9	(a)	(a)	14.57	3.34	(a)
Do.	12	4.97	12.03	5.21	(a)	(a)
Average				9.89		
Rice hulls, treated 1.5 per cent NaOH	9	23.72	20.28	35.04	51.36	13.87
Do.	11	34.60	36.07	41.04	60.43	18.06
Average		29.16	28.43	38.04	55.92	15.97
Rice hulls, treated 3 per cent NaOH	18	34.02	28.42	50.53	48.91	10.97
Do.	19	33.96	22.71	39.57	29.27	21.73
Average		33.99	25.57	45.05	39.09	16.35
Increase due to treatment (1.5 per cent NaOH)		24.19	16.40	28.15	52.58	15.97
Percentage increase		486.72	135.76	254.63	1,074.25	(c)
Increase due to treatment (3 per cent NaOH)		29.02	13.49	35.16	35.75	16.35
Percentage increase		583.90	111.07	355.51	1,770.36	(c)
Cottonseed hulls, untreated	18	46.06	54.00	54.74	92.26	(a)
Do.	19	59.08	62.41	62.65	88.10	31.26
Average		53.02	58.21	58.70	90.18	
Cottonseed hulls, treated 1.5 per cent NaOH	18	51.82	51.29	66.84	108.87	(a)
Do.	19	57.21	53.83	68.53	92.70	9.45
Average		54.52	52.56	67.69	100.78	
Increase due to treatment		1.50	d 5.65	8.99	16.60	d 21.81
Percentage increase		2.83	d 9.71	15.32	11.75	d 69.77
Flax shives, untreated	18	(a)	(a)	e 3.92	e 6.91	(a)
Do.	19	30.15	18.67	33.18	25.36	27.79
Flax shives, treated 1.5 per cent NaOH	18	18.31	20.15	31.34	39.09	(a)
Do.	19	39.21	25.37	45.43	46.32	20.32
Average		28.76	22.76	38.38	42.71	
Increase due to treatment		d 1.41	4.09	5.20	17.35	d 7.47
Percentage increase		d 4.61	21.91	15.67	68.41	d 26.88

a Negative.

b For some reason sheep 16 did not digest the oat hulls fed in this trial at all well. As it is not wise to average results which vary so widely as do those for these two sheep on this material, the figures for sheep 16 in this trial are not made use of in subsequent calculations.

c Can not be computed; no basis to start from, as digestibility of lignin in the untreated material was nil.

d Decrease.

e It would be unwise to average these results. The figures for sheep 19 are employed in the subsequent calculations.

Where varying strengths of NaOH were used on the same material, an increase in strength of solution was almost invariably accompanied by a considerable increase in digestibility of the hulls, most marked when the comparison was between 1 per cent NaOH and 1.5 per cent NaOH, not so marked where 3 per cent NaOH was used. In this connection it should be noted that losses in weight due to treatment were slightly increased by an increase in strength of the soda.



TABLE VIII.—*Effect of varying strengths of sodium hydroxide in increasing digestibility of fibrous material<sup>a</sup>*

Solution used.	Percentage increase over untreated hulls.				
	Total dry matter.	Crude fiber.	N-free extract.	Pentosans.	Lignin.
1 per cent NaOH (oats).....	94.17	57.45	89.76	97.91	252.89
1 per cent NaOH (barley).....	14.54	16.72	25.00	36.90	84.97
1.5 per cent NaOH (oats).....	122.43	73.24	131.92	105.46	252.68
1.5 per cent NaOH (barley).....	37.57	81.47	41.35	75.19	(b)
1.5 per cent NaOH (rice).....	486.72	135.76	284.63	1,674.25	(c)
3 per cent NaOH (rice).....	583.90	111.67	355.51	1,170.36	(c)

<sup>a</sup> The cottonseed hulls and flax shives are not included in this summary because of the negligible effect the soda solution had on their digestibility.

<sup>b</sup> Negative.

<sup>c</sup> Averages not available for lignin because of varying results in its digestibility.

As a rule, increases in the relative amount of a component due to treatment were accompanied by an increase in its percentage digestibility.

Taking into consideration both the loss in weight and the increased digestibility due to the action of the soda (see Tables IV and VI) we obtain net gains as shown in Table IX.

TABLE IX.—*Average net gain in total digestible dry matter and in the important digestible nutrients of oat hulls and barley hulls on the basis of 100 pounds of dry matter treated*

Material.	Total digestible dry matter.	Digestible crude fiber.	Digestible N-free extract.	Digestible pentosans.	Digestible lignin.
Oat hulls, treated 1 per cent NaOH.....	7.72	5.47	3.60	11.81	<sup>a</sup> 7.06
Oat hulls, treated 1.5 per cent NaOH.....	31.04	<sup>a</sup> 19.14	11.59	15.88	2.94
Barley hulls, treated 1 per cent NaOH.....	<sup>b</sup> 4.55	2.81	<sup>b</sup> 3.68	4.81	1.97
Barley hulls, treated 1.5 per cent NaOH.....	5.79	8.18	11.31	8.46	(c)

<sup>a</sup> Referring to Tables IV and VI it will be seen that the total crude fiber and total lignin of oat hulls each showed a net gain instead of a net loss after treatment. As already noted, this is impossible, so the results in question are obtained by assuming that no loss of these constituents was involved.

<sup>b</sup> This result represents a net loss.

<sup>c</sup> Digestibility of lignin in this instance was a minus quantity.

Considering the net gains as shown in the table, it can be said that with one exception (barley hulls, 1 per cent NaOH) the increase in digestibility outweighed the loss by solution in the soda. It is questionable, however, whether in those materials showing the smaller gains the increase would offset the cost of treatment. The extra 0.5 per cent of sodium hydroxide apparently makes a great difference in the final result.

The barley hulls probably would have made a more favorable comparison with the oat hulls had they not contained so much starchy material. Unfortunately, the separation of the endosperm from the hull had not been nearly so complete as in the case of the oat hulls (due in part, probably, to the greater tenacity with which the barley hull clings to the endosperm, or possibly to a less perfect mechanical method of separation), and as a result there was quite a large amount of the floury portion of the grain adherent to the hulls.

On a dry-matter basis the digestible matter in a ton of oat hulls was increased by treatment with 1.5 per cent NaOH from 725 pounds to 1,345

pounds, the digestible crude fiber from 349 pounds to 732 pounds, and the digestible nitrogen-free extract from 390 pounds to 622 pounds. The digestibility of the pentosans, which are distributed between the fiber and nitrogen-free extract, was increased from 287 pounds per ton to 605 pounds. In short, the feeding value of the oat hulls was about doubled. It seems that such a result should warrant further investigation with the idea of making the process of economic importance.

#### SUMMARY

This paper reports the results of an investigation on the problem of increasing the digestibility and feeding value of grain hulls.

A review of the literature shows:

1. That the important constituent of the cereal and other straws is lignocellulose, a compound the chemistry of which is not fully understood. It is known, however, that it consists of cellulose linked in some manner with lignin and that the presence of the latter compound is characterized by the splitting off of methoxy ( $\text{CH}_3\text{O}$ ) groups upon hydrolysis.
2. That the action of dilute alkali on fiber is threefold, consisting of separation of the silicic acid which forms a part of the incrusting material, splitting off of the methoxy groups of the lignin with formation of acetic acid, and springing of the bonds which exist between the lignin and cellulose.
3. That practically all of the work on this problem has been carried on in Germany, most of it since the commencement of the World War, and the material most generally investigated has been straw, the digestibility of which has been decidedly increased. Grain hulls do not appear to have been worked with heretofore.

In our investigation the method used for treatment of the hulls was that of Beckmann. The materials treated were oat hulls, barley hulls, rice hulls, cottonseed hulls, and flax shives. The hydrolyzing agent was cold dilute sodium hydroxid, the strengths employed being 1, 1.5, and 3 per cent.

Most satisfactory results were obtained with NaOH of 1.5 per cent strength; 1 per cent was apparently too dilute, and 3 per cent in the one instance used did not have sufficient increased action over the 1.5 per cent to warrant its use.

The effect of the alkali on the composition of the hulls was ascertained by the usual methods of fodder analysis, supplemented by determination of some of the ultimate constituents of the hulls both before and after treatment.

The result of treatment was a decrease in all proximate constituents except the crude fiber, which from an absolute standpoint remained practically the same but relatively was considerably increased.

Losses in weight due to the treatment were noticed in all materials; they were greatest in the case of the flax shives (25 per cent), and least in the case of the cottonseed hulls (9.7 per cent).

The effect of the alkali on the digestibility of the hulls was ascertained by the usual methods employed in digestion experiments, sheep being the animals used. As a result of the treatment with 1.5 per cent NaOH the digestibility of the important constituents of oat hulls and barley hulls was markedly increased, the feeding value of the oat hulls being doubled. The digestibility of rice hulls was also improved greatly, but not sufficiently to be of economic significance. The results with cottonseed hulls and flax shives were in the main negative.

Since the digestibility of oat and barley hulls is greatly improved by the action of dilute alkali, some method should be devised that could be applied on an economic scale. Also, a method for the improvement of the digestibility of cottonseed hulls is worthy of further attention.

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# THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F<sub>1</sub> HYBRID<sup>1</sup>

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## INTRODUCTION

This paper has a twofold purpose: (a) The presentation of the results of an investigation of the physicochemical properties of the leaf tissue fluids of Egyptian and Upland cotton as grown under irrigation at Sacaton, Ariz.; (b) a comparison of the properties of the leaf tissue fluids of the F<sub>1</sub> hybrid between these two cottons with those of the two parent types.

The consideration of both of these groups of problems rests on the results of much antecedent work.

In a series of investigations carried out in natural plant habitats the writers have shown that there is a close relationship between the aridity of the habitat and the osmotic concentration of the plant tissue fluids of the native vegetation. These results, obtained by careful cryoscopic measurements in the southwestern deserts (30)<sup>2</sup>, in the mesophytic habitats of the Eastern United States (29), in the Jamaican deserts (26) and rain forest (28), in the mangrove swamp (27), and in many other localities for which the data are not yet published, fully substantiate and greatly extend the general conclusions drawn from the earlier plasmolytic determinations by Drabble and Drabble (14) and by Fitting (15). That the osmotic concentration of the plant tissue fluids is a factor of importance in determining the capacity of the plant for survival under conditions of aridity is suggested by work on the relationship of the loranthaceous parasite to its host, as investigated by plasmolytic studies by Senn (48), and by cryoscopic studies on both rain forest and desert (20, 25, 33).

Concurrently with these investigations on the osmotic concentration and electrical conductivity of plant tissue fluids, another group of workers, among whom Wherry has been the most active, has furthered investigations on the relationship between soil acidity and plant distribution. In this place it will suffice to refer to the more recent reviews by Wherry (51) and by Atkins (2).

From the earliest stages of the investigations we realized the desirability of investigations of the sap properties of agricultural plants. Since natural vegetations have been shown to differ in the physicochemical properties of their tissue fluids, it would seem quite reasonable to assume

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<sup>2</sup> Reference is made by numbers (italic) to "Literature cited," p. 325-327.

that the properties of the tissue fluids of crop plants may be of importance in determining the possibility of their growth and productiveness under the varying soil and atmospheric conditions of different regions.

This assumption (which receives additional support from the work which has been done on native vegetation as an indicator of the suitability of land for crop production, as developed by Shantz (49), by Kearney, Briggs, Shantz, McLane, and Piemeisel (41), and by Clements (11) seems so reasonable that it becomes of the greatest importance to determine to what extent species and varieties which are of agricultural and horticultural importance are differentiated with respect to the physicochemical properties of their tissue fluids, and to ascertain in how far observed differences in these properties represent innate and relatively constant differences and to what degree they are the resultant of varying environment conditions, such as dryness and salinity of soil, insolation, and the evaporating power of the air.

From the beginning of these investigations<sup>3</sup> we also foresaw the possibilities of a consideration of the relation of the physicochemical properties of the plant tissue fluids to the relative vigor of hybrid and parental forms and to other problems in genetics. For the past several years we have, therefore, been seeking the materials and opportunities for an investigation of the physicochemical properties of the tissue fluids of heterozygous individuals as compared with those which are homozygous for one or many characters. It seemed desirable if possible to obtain parent forms which themselves differed in respect to such tissue-fluid properties as osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration.

Cotton as grown in Arizona and California is of interest to the physiologist because it is of necessity grown on irrigated land where the salt content of the soil may be relatively high and because it is a plant with heavy and watery foliage, which must nevertheless be grown under the conditions of temperature and insolation of a fairly severe desert climate. Both of these factors—economic importance and peculiar conditions of growth—combine to render of special importance the investigation of any physiological peculiarities of the plant which may influence its capacity for growth under the rigorous conditions of the Southwest.

## HISTORICAL

The earlier literature of Egyptian cotton as grown in Egypt has been summarized, or at least cited, by Balls (4), who has more recently considered certain problems of growth and yield (7). We make no attempt in this place to review the purely agronomic literature on American cultivation of Egyptian cotton, much of which is cited in a paper describing the American-Egyptian cotton industry, by Scofield, Kearney, Brand, Cook, and Swingle (47). Mention should be made also of the recent investigations of King (43) on water-stress behavior of Pima

<sup>3</sup> In 1920, Dr. T. H. Kearney, physiologist in charge of alkali and drought-resistant plant investigations, Bureau of Plant Industry, and Mr. G. N. Collins, botanist in charge of biophysical investigations, Bureau of Plant Industry, recognizing the possible agricultural bearing of the results already obtained from the studies of native vegetation, invited us to undertake investigations on the physicochemical properties of native indicator plants and of crop plants in the arid West.

These field operations have included studies on the native vegetation of Tooele Valley, Utah, of cereals grown under dry farm agriculture at Nephi, Utah, and on Egyptian and Upland cotton grown under irrigation at Sacaton, Ariz. While all these studies in the arid West are mutually supplementary, and should be considered in their entirety, they must be presented in sections. The present paper deals exclusively with a comparison of Egyptian and Upland cotton and their F<sub>1</sub> hybrid.



Egyptian cotton in Arizona and of the investigation of cross- and self-fertilization as related to the maintenance of purity of strains by Kearney (39). These furnish a key to the more important literature. The contents of a number of chemical papers on the cotton plant do not interest us in this connection.

Limiting our attention to the very meagre literature of the relationship of the cotton plant to soil salinity and dryness, we may note that 20 years ago Kearney and Means (42) found that in fields in the neighborhood of Alexandria, Egypt, where the washing used in reclamation had not yet been completed, the salt content of the upper 2 feet of the soil was, among good plants, 0.6 per cent; near occasional plants in partly bare ground, 1.8 per cent; on wholly bare ground, 2 per cent and higher.

"These figures indicate an exceptionally high degree of resistance in the cotton plant, marking it as one of the very foremost in this respect of the world's great crops." Further nonquantitative notes on the occurrence of cotton on saline land are given.

Finally, Kearney (36) has discussed the growth of Egyptian cotton on alkali soils. His results indicate that with alkali of the type found at Sacaton the fruitfulness of the plants is likely to be impaired when the salt content exceeds 0.4 per cent of the dry weight of the soil. This, he concludes, would seem to be about the limit for profitable production of this crop in the presence of alkali of the type found at Sacaton, although he observed that the quality of the fiber does not necessarily suffer in the presence of 0.55 per cent of salt. He found that the moisture capacity of the soil is an important factor in determining the size, vigor, and fruitfulness of Egyptian cotton plants. The alkali resistance of Egyptian cotton is relatively high when other conditions are favorable. It would appear that a fair yield of fiber of good commercial quality can be obtained when nearly one-half of 1 per cent of the total dry weight of the soil consists of readily soluble alkali salts, provided that carbonates are absent or form only an inconsiderable proportion of the total mineral solutes.

The only literature on the salt content of the Egyptian cotton plant of which we are aware consists of two short papers by Balls (5, 6), both of which came to our attention after this manuscript was practically completed.

In the first of these (5) he calls attention to a chance result which indicates that the tree cottons and Egyptian cotton bring up from deeper layers and deposit on the surface of the soil through the shedding of their leaves sufficient quantities of substances, of which NaCl is one constituent, to render the soil unsuitable for other plantings.

In the second (6) he compares the chlorid content of three pure strains of Egyptian cotton grown at Gizeh, Egypt, on land which contained not more than 0.1 per cent of NaCl in the surface layers, even after long deprivation of water. From these analyses, which are few in number and based on the dry weight of the leaves only, he concludes that Egyptian cotton growing under typical field crop conditions has a salt content which indicates a concentration of 0.3 per cent NaCl in the leaf cell sap. This concentration varies with the salinity of the soil, though not proportionally. It also varies with the particular pure strain or variety. Plants of two Egyptian strains growing with interfering root systems may show differences of as much as 10 : 7 in the salinity of their cell sap. This fact, he suggests, may have some utility in the breeding of strains for the salty lands of the northern delta of Egypt.

In conclusion, Ball makes the significant suggestion: "Egyptian cotton (*Gossypium peruvianum*) may be classified as a facultative halophyte."

As far as we are aware the only previous work on the hydrogen-ion concentration of the tissue fluids of cotton is the single determination of  $P_H$  4.6 in leaf, stem, and petal tissue given by Atkins (x). As will be shown later, this represents a distinctly higher acidity than that which we have found in any instance in the leaf tissue fluids of cotton.

#### MATERIALS AND METHODS

Since we are here dealing with a problem in which every possible source of error must be considered, and in as far as possible eliminated, in order to secure conclusive results, we shall describe the materials and methods employed in some detail. The importance of these details is not, we believe, overemphasized.

A detailed discussion of the Egyptian and Upland types of cotton and of their  $F_1$  hybrid is not necessary in this place in view of the fact that the results of an investigation of the hybrids which has been in progress for the past several years have been published (40). The data given by Kearney in regard to height of plants and dimensions of the leaves were confirmed by measurements made on the material used by the writers in their investigations in 1921. It need only be mentioned here that the Egyptian and Upland types differ in a large number of morphological characters, the differences being of a kind and degree indicating that they belong to very distinct botanical species. When the two types are grown under comparable conditions the Egyptian plant is taller and has longer internodes and branches and larger, more deeply lobed, thicker, smoother, and darker green leaves than the Upland plant.

#### GENETIC NATURE OF THE SEED EMPLOYED, AND CHARACTERISTICS OF THE VARIETIES AND OF THEIR $F_1$ HYBRID

Three varieties of cotton are considered in this paper: Pima represents the Egyptian, while Acala and Meade belong to the Upland group of cottons.

The following is the history of the Egyptian and Upland seed employed for the special cultures upon which the physicochemical determinations were made in 1921. The origin and characteristics of the Pima variety have been described by Kearney (37, 40). The "Pima selfed" seed was obtained by selfing a number of plants in a progeny designated Pima  $H_1-5$  Acala A, which was grown at Sacaton in 1920. This progeny was derived, by three generations of controlled self-pollination, from progeny  $P_1-40$  of 1917, which had been derived by three generations of controlled self-pollination, from plant  $P_1$ , selected in 1914. The line of descent of  $P_1-40$  has been described elsewhere (38). The "Pima selfed" material of this experiment represented, therefore, seven generations of strict inbreeding and was presumably approaching homozygosity.

The origin and characteristics of the Meade variety are described by Meloy and Doyle (46).

The "Meade selfed" seed was secured by self-fertilizing a few plants of Meade cotton grown at Sacaton in 1920. These were grown from seed harvested from experimental plantings in Georgia, conducted under the direction of Dr. O. F. Cook, of the Office of Acclimatization and Adaptation Investigations of the Bureau of Plant Industry. This

seed came from isolated cultures of naturally pollinated (not selfed) plants. Mr. G. S. Meloy, who furnished the seed, considers it pure as far as varietal characters are concerned. It is not, however, as closely inbred as is the "Pima selfed" of the experiment.

The "Pima bulk" of the experiment was grown from seed from a selected but not strictly inbred strain. While unquestionably pure Pima, it was not of controlled, self-fertilized, ancestry.

The Meade bulk and the Acala<sup>4</sup> bulk used in the planting were both from lots of seed sent to Sacaton by Mr. Meloy in 1919 and held over until 1921. Both are supposed to be varietally pure, although not resulting from controlled inbreeding.

The  $F_1$  hybrid was derived as follows: The Pima parent of the  $P \times M F_1$  used was a plant representing the sixth inbred generation in progeny Pima H1-2A of 1920, a sister progeny of PH1-5 Acala A which furnished the "Pima selfed" seed in the experiment.

The Meade parent of the  $P \times M F_1$  was a plant grown at Sacaton in 1920 from seed obtained from the experimental plantings in Georgia. This plant was one of a lot grown from seed produced by naturally pollinated (not selfed) flowers in an isolated planting of this variety in Georgia, and was considered "pure" by Mr. Meloy, who sent it to Sacaton.

Flowers were selfed on a few individuals in the 1920 planting at Sacaton, and these furnished the "Meade selfed" seed planted in the physico-chemical experiments of 1921. Thus the Meade parent of this hybrid was by no means so closely bred as the Pima parent.

It may be noted that the seed used was not of exactly the same age for all of the cultures. This difference in age probably does not influence the physiological characteristics of the plants in any way, since the cotton seed is very long lived.<sup>5</sup>

The  $F_1$  hybrids between Egyptian and Upland cotton show intensification in most size characters. In respect to other characters the hybrid mean may be intermediate or may approximate that of one or the other parent. The  $F_1$  is highly uniform. The greater vegetative vigor is conspicuously shown by the accompanying plate (Pl. 1), in which the  $F_1$  hybrid is shown between the Egyptian and the Upland parental types.

#### CULTURAL METHODS

In preparing the planting scheme it seemed desirable to grow the plants under the standard conditions of the Cooperative Testing Station experiments with the cottons. This involved the use of an irrigation border 26.5 feet in width by 400 feet in length. This accommodated seven rows of cotton 3.5 feet apart, with plants 1 foot apart in the rows. In order to avoid the influence of soil heterogeneity, which has been shown to be an important factor in crop yields (21, 32), and which may reasonably be assumed to be an important factor in determining the physico-chemical properties of the tissue fluids of the plant organism in regions

<sup>4</sup> Acala is an Upland variety of Mexican origin, introduced by G. N. Collins and C. B. Doyle, and adapted by selection to conditions in the United States by O. F. Cook and his colleagues. Acala is a big-bolled type, producing fiber from  $1\frac{1}{4}$  to  $1\frac{1}{2}$  inches long.

<sup>5</sup> It has been found that Pima seed which had been stored at Sacaton during nine years germinated readily when planted. Moreover, the stock of Pima planting seed distributed to farmers in the Salt River Valley in the spring of 1921 had been held over one year at Tempe. In order to make sure that its viability was unimpaired, the germination was thoroughly tested by the seed laboratory of the Bureau of Plant Industry just before the seed was distributed, and the average germination percentage proved to be exactly the same as that for the same stock of seed shortly after it was harvested. The seed was approximately one year older when the second series of germination tests were made. There is a possibility that seed of the Upland cottons may lose its viability more rapidly, but this is improbable.

in which the soil is rich in inorganic solutes, it seemed essential to distribute the Egyptian and Upland cottons and their  $F_1$  hybrids uniformly over the field.

The plan adopted is shown in figure 1. Here the heavy line indicates Pima Egyptian, the broken line indicates Meade Upland, the light solid line indicates Acala Upland, and H denotes a hybrid plant. Beginning at the south end of the field, 10 feet of each row was planted to Pima cotton. On four of the rows (1, 3, 5, and 7) one hill of hybrid was planted. On the first and fifth row self-fertilized Pima and Meade seed was used. For the other rows bulk seed was employed.

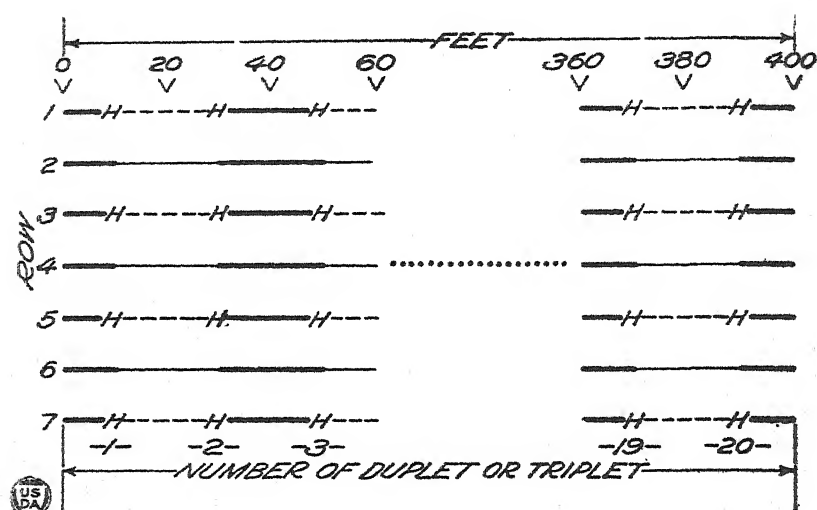


FIG. 1.—Planting scheme for comparison of Upland and Egyptian cottons and their  $F_1$  hybrid. Only the two ends of the plot are shown. The heavy solid line represents plants of Pima Egyptian, the broken line plants of Meade Upland, the light solid line plants of Acala Upland, and the letter H an  $F_1$  hybrid plant. The term duplet is used to designate plants of Egyptian and Upland which are contiguous and in the same row. The term triplet designates an  $F_1$  plant (H) having Egyptian plants on one side and Upland plants on the other, all in the same row.

The Pima cotton and the hybrid, when it occurred, was then followed by 20 feet of Upland cotton, which was followed by 20 feet of Pima on rows 2, 4, and 6, and by a hybrid plant, and then by slightly less than 20 feet of Pima on rows 1, 3, 5, and 7. Thus the length of the plot afforded space in each row for 9 subrows, each of 20 feet, and for 2 half-subrows, each of 10 feet, of Pima cotton, and for 10 subrows, each of 20 feet, of Upland cotton. In the rows which contained hybrid plants the space for the Pima and Upland subrows was slightly shortened to make room for the hybrid hills. The spacing in the rows of the individual plants of all varieties and hybrids was identical.

This plan distributed plants of all types to be compared uniformly over the entire field. Collections were made at the 20 points of contact of Egyptian and Upland cotton or of Egyptian, hybrid, and Upland cotton in each of the rows. The hybrid plant and the contiguous Egyptian and Upland plants are designated as a triplet. The contiguous plants of Egyptian and Upland cotton in the rows containing no hybrid individuals are conveniently designated as a duplet.

The cultural technique was that generally employed for the growth of Egyptian and Upland cotton at the Cooperative Testing Station at Sacaton. Since all the materials considered were grown under as nearly as possible identical conditions, it seems unnecessary to detail these methods here.<sup>6</sup>

#### COLLECTION OF SAMPLES

Cotton is not in its morphological features a desert plant. It wilts readily and must be copiously supplied with irrigation water in order to produce a crop under the intense heat and scanty precipitation of the Southwest. During August the plants and the condition of the soil in which they are growing are continually varying, not merely from day to day but literally from minute to minute. Immediately after irrigation, in which the whole surface of the "border" is flooded with a sheet of water, the soil is fully saturated with water, but this is lost very rapidly. Water loss from the plants through transpiration increases very rapidly after sunrise, and great care must be taken not to obtain samples of leaves which have already begun to wilt. When there is a heavy dew in the morning, as occasionally occurs after the summer rains have begun, it is difficult to secure samples in the limited period between the time when the leaves have scattered droplets of external moisture and the time when they begin to wilt under the influence of the intense insolation.

Because of the continual changes in the condition of the plants, the taking of the samples is one of the most exacting features of the work. In addition to the care necessary in selecting the brief period most suitable for taking the samples, two possible sources of error had to be carefully avoided.

There must be introduced no artificial source of differentiation between the two species under consideration, or between the species and their hybrid, through the taking of the samples from different portions of the field or at different times.

The leaves taken from the plants to be compared must be comparable in maturity.

Artificial differentiation between the species or between the species and their hybrid may be avoided in cases in which the varieties to be compared belong to the same duplet (rows 2, 4, and 6) or the same triplet (rows 1 and 5 and rows 3 and 7) by making sure that collections are taken simultaneously from the two or three members of the duplet or triplet.

In the first series of collections (1920) it was possible to avoid the influence of heterogeneity in the soil conditions<sup>7</sup> because of the fact that in a number of breeding experiments under way at Sacaton, Pima cotton and Upland cotton varieties were grown side by side in the same plot or "border." All collections were taken from adjacent rows or from closely associated plants in the same row. In the investigations of 1921 the possible influence of this factor was precluded by a special planting scheme, as described above.

To avoid the possible influence of time variations, the samples from the two or three members of the duplet or triplet were taken at as

<sup>6</sup> We are greatly indebted to Mr. Walter F. Gilpin, who carefully made the plantings, took some of the records and facilitated our work in other ways.

<sup>7</sup> Readings made by Mr. Kearney with the soil bridge on samples of soil taken in the neighborhood of the various sets of tissue samples, indicated differences in the salt content from place to place in the field.

nearly as possible the same time. Probably not more than five minutes elapsed in any case between the sampling of any two members of a group. Generally the time was much less than this. Probably no appreciable source of error was introduced in this way.

In the case of the loss of any sample through breakage, contamination, or through any discernible evidence of an erroneous reading, new samples of both members of the duplet or of all three members of the triplet were taken before the completion of the whole series of determinations.

Rows 1 and 5 were planted to self-fertilized Pima and self-fertilized Meade. Rows 3 and 7 were planted to Pima and Meade grown from bulk seed. Thus it is possible to consider in a preliminary way whether there are differences between the sap properties of these two classes (those grown from self-fertilized and those grown from open-fertilized seed) of plants. Again, rows 1, 3, 5, and 7 were planted to Pima Egyptian and to the Upland variety Acala. Thus it is possible to compare the two varieties of Upland cotton in a wholly preliminary way.

If the samples had been taken systematically across the field, it is evident that changing conditions from day to day might be a source of large differences between the several rows. The collections were, therefore, made from duplets or triplets selected as nearly as possible at random from different portions of the field.

In order to avoid in so far as possible differences due to age, collections were made primarily of matured leaves from the main stem or the largest branches of the plants. Great care was taken to select perfect leaves.

A possible source of variation in the constants is personal equation in the making of the collections. The taking of the samples presented considerable difficulty because of the limited time in which it was necessarily carried out in order to have environmental conditions as nearly comparable as possible. Three workers, therefore, took part in collecting the material.

In the first series of collections the samples were taken by two workers, and notes were taken by the third. Had one of the two workers taken either Egyptian, Upland, or hybrid samples continuously, it seems quite possible that differences in personal equation might have influenced the results. But the two collectors generally alternated in the variety collected. Thus, any systematic influence of personal equation was avoided.

It is evident, however, that if there be a real personal equation on the part of the different collectors, this will tend, in the system of collection adopted for the first series, to obscure the difference between the forms.

To avoid this possibility, a modification of the plan was adopted in the second series of collections. One worker, who had enjoyed longer experience in botanical work than the others, collected all the leaves used for samples. Thus the possibility of an influence of personal equation in the collection of the samples would seem to have been practically, if not entirely, eliminated in the second series.

#### 4. ANALYTICAL METHODS

The samples of tissue were placed at once in rubber-stoppered glass tubes and in a very short time were in a cooling mixture for freezing antecedent to the extraction of the sap, in accordance with the findings of

Dixon and Atkins (12) and ourselves (16, 17). Freezing-point lowering,  $\Delta$ ; specific electrical conductivity,  $\kappa$ ; and hydrogen-ion concentration, expressed as  $P_H$ , were then determined on the centrifuged sap, extracted from the tissue by pressure.

In the first series of determinations (see p. 283) the physical measurements were made as soon as possible after the extraction of the leaf fluids. In the second series the expressed sap was allowed to stand overnight in the ice box.

Freezing point lowering was measured by means of a mercury thermometer graduated to hundredths of degrees. Electrical conductivity was determined at 30° in Freas conductivity cells standardized against  $N/10$  KCl, considered as having a conductivity of 0.01412 reciprocal ohm at 30°. The Washburn half-meter bridge with extension coils, giving 10 M. of wire, and a Leeds and Northrup resistance box was used.

Hydrogen-ion concentration was measured by means of the Wendt modification of the Hildebrand assemblage. In this method a calomel cell charged with normal KCl, a small Weston Millivoltmeter and a sliding resistance of 500 ohms with current capacity of one ampere were used. Hildebrand, Sharp, Hoagland, and others (10) have recognized the limitations of precision of this method. The accuracy of the physical measurements is, however, sufficient for the comparisons which are emphasized in this paper.

In discussing these acidities we must not forget that the determinations were made on sap extracted by pressure from killed tissues. Haas (18) has stated that a considerable change in reaction may take place in the cell as it dies (from  $P_H$  3 to  $P_H$  7), but he evidently refers to the changes taking place in the natural ageing and death of the cells, rather than to errors introduced by the killing of the cell and the extraction of the cell sap. His own determinations were made by means of extracts obtained by rapidly macerating petals in a mortar with the solvent. McClendon and Sharp (44) noted an increase in the acidity of freshly expressed carrot juice on exposure to the air for a short time. They also give some data on  $P_H$  of fluids extracted from boiled and unboiled tissues. Finally, Atkins (1) has noted a similar increase in the acidity of expressed sap on exposure to the air, and suggests a coordination of this result with those obtained by Bunzell (8) on acidity and oxidase activity. It is also possible, as noted by Hoagland and Davis (35), that extracellular substances in the tissues may influence the concentration or reaction of the cell sap after the crushing of the tissues.

Whatever may be the results of further investigations on the relative values of the hydrogen-ion concentration of extracted sap as compared with that of the sap occurring in the tissues, the facts remain (a) that the only method by which the problem of hydrogen-ion concentration may be investigated in a series like the present is upon extracted sap, (b) that the technique used introduces no artificial differences between the biological series considered, and (c) that, as will be shown below, the results for the various series considered here show a high degree of consistency, thus indicating that there is no highly variable disturbing factor in the technique.

The results in the accompanying tables are expressed in terms of freezing-point depression,  $\Delta$ , corrected for the amount of ice separating on under-cooling. Those who prefer to consider the results in terms of atmospheres may transmute the values of freezing-point lowering given



in this paper into atmospheres by the use of a published table (24), as we have done in a few cases.

Conductivities have been expressed in reciprocal ohms or mhos.

At the time of making these determinations we were unacquainted with the methods suggested by Mason (45) for estimating the influence of viscosity upon electrical conductivity. The conductivities given are, therefore, the raw values wholly uncorrected for the resistance of the sap. Inasmuch as the biological forms considered do not show large percentage difference in freezing-point depression, this fact probably does not invalidate the constants to a measurable degree for the purpose in hand.

We have felt it desirable to express in some manner the relative value of the electrical conductivity.

While Chandler (9), Dixon, and Atkins (13), and more recently Mason (45), have attempted to differentiate between electrolytes and non-electrolytes by expressing the conductivity in terms of the osmotic concentration which would be produced by a solution of a specific solute (KCl) of the same electrical conductivity as the sap, it has seemed to us wisest to limit ourselves, as in earlier papers, to the purely numerical ratio of one constant to the other. We have, therefore, merely employed the ratio of the specific electrical conductivity to the freezing point depression as a measure of the relative concentration of dissociated ions and total solutes (both dissociated and nondissociated electrolytes and nonelectrolytes). It is impossible to attach any specific chemical meaning to the ratio  $\kappa/\Delta$ . It is, however, useful for purposes of comparison.

The values of hydrogen-ion concentration have been calculated by the formula

$$P_R = \log \frac{1}{[H]} = \frac{E' - E}{0.0001983 T}$$

where  $E'$  is the electromotive force in millivolts of the tissue fluid under investigation as read from the voltmeter,  $E$  is the E. M. F. of the KCl calomel electrode employed (taken as  $E = 0.2828$  volt for the normal KCl electrode), and  $T$  the absolute temperature.<sup>8</sup>

#### STATISTICAL ANALYSIS OF DATA

In the analysis of the series of measurements it has been necessary to employ to some extent the methods of the modern higher statistics. The simpler biometric notation is now familiar to most biologists. When special methods are used, formulas are given.

#### PRESENTATION AND ANALYSIS OF DATA FOR THE COMPARISON OF PARENTAL TYPES

Because of the large number of factors which might influence the results of physicochemical measurements on the tissue fluids of a crop plant such as cotton, and because of the fact that the varieties considered in 1920 and 1921 are not in all cases the same, it seems desirable to present the results of the preliminary investigation carried out by two of us in 1920, as well as the results of the more carefully planned investigation of 1921.

<sup>8</sup> We are deeply indebted to Mr. C. J. King, who encouraged us to monopolize practically the whole of his laboratory space while our work at Sacaton was in progress and facilitated our work in every way in his power.



## PRELIMINARY STUDY IN 1920

A first comparison was based on Pima Egyptian and Acala Upland cotton taken August 14, 1920. The plants from the north and south ends of this border differed greatly in size, apparently because of the influence of alkali. Samples 1 and 2 were from well-grown plants from the south ends of the border. Samples 3 and 4 were from somewhat dwarfed plants at the north end of the border.

TABLE I.—*Physicochemical constants for the leaf tissue fluids of various types of cotton grown in 1920, and the differences between the constants for the various types*

Types and varieties compared.	Sample numbers.	Depression of freezing point, $\Delta$ .	Osmotic concentration in atmospheres, P.	Specific electrical conductivity, $\kappa$ .	Ratio of conductivity to depression, $\kappa/\Delta$ .
Pima Egyptian.....	(1)	1.392	16.74	0.03880	0.02787
Acala Upland.....	(2)	1.218	14.65	0.03083	0.02532
	(1)-(2)	+0.174	+2.09	+0.00797	+0.00255
Pima Egyptian.....	(3)	1.573	18.91	0.03490	0.02219
Acala Upland.....	(4)	1.349	16.23	0.02635	0.01953
	(3)-(4)	+0.224	+2.68	+0.00855	+0.00266
Pima Egyptian.....	(5)	1.244	14.97	0.03071	0.02468
Acala Upland.....	(6)	1.201	14.45	0.02871	0.02392
	(5)-(6)	+0.043	+0.52	+0.00200	+0.00076
Pima Egyptian.....	(7)	1.390	16.72	0.03152	0.02266
Sea Island.....	(8)	1.193	14.35	0.02930	0.02456
Acala Upland.....	(9)	1.274	15.33	0.03040	0.02386
Kekchi Upland.....	(10)	1.178	14.17	0.02939	0.02496
	(7)-(8)	+0.197	+2.37	+0.00222	-0.00190
	(7)-(9)	+0.116	+1.39	+0.00112	-0.00120
	(7)-(10)	+0.212	+2.55	+0.00213	-0.00230
	(8)-(9)	-0.081	-0.98	-0.00110	+0.00070
	(8)-(10)	+0.015	+0.18	-0.00009	-0.00040
Pima Egyptian.....	(11)	1.432	17.22	0.03184	0.02224
Wa Gale Asiatic.....	(12)	1.169	14.06	0.02839	0.02430
	(11)-(12)	+0.263	+3.16	+0.00345	-0.00206
Pima Egyptian.....	(13)	1.285	15.46	0.04781	0.03721
Lone Star Upland.....	(14)	1.058	12.74	0.03612	0.03415
	(13)-(14)	+0.227	+2.72	+0.01169	+0.00306
Pima Egyptian.....	(15)	1.123	13.51	0.03661	0.03260
Lone Star Upland.....	(16)	0.943	11.35	0.03188	0.03471
	(15)-(16)	+0.180	+2.16	+0.00473	-0.00211
Pima Egyptian.....	(17)	1.334	16.05	0.03461	0.02594
Lone Star Upland.....	(18)	0.974	11.72	0.02788	0.02864
	(17)-(18)	+0.360	+4.33	+0.00673	-0.00270
Pima Egyptian.....	(19)	1.154	13.89	0.02971	0.02575
Durango Upland.....	(20)	1.030	12.39	0.02656	0.02580
	(19)-(20)	+0.124	+1.50	+0.00315	-0.00005
Pima Egyptian.....	(21)	1.254	15.09	0.03190	0.02543
Durango Upland.....	(22)	1.230	14.80	0.03118	0.02536
	(21)-(22)	+0.024	+0.29	+0.00072	+0.00007
Pima Egyptian.....	(23)	1.162	13.98	0.03163	0.02724
Durango Upland.....	(24)	1.055	12.70	0.02861	0.02712
	(23)-(24)	+0.107	+1.28	+0.00302	+0.00012
Pima Egyptian.....	(25)	1.347	16.20	0.05574	0.04137
Holdon Upland.....	(26)	1.250	15.04	0.05154	0.04123
	(25)-(26)	+0.097	+1.16	+0.00420	+0.00014
Pima Egyptian.....	(27)	1.253	15.07	0.03209	0.02561
Acala Upland.....	(28)	1.113	13.40	0.02794	0.02510
	(27)-(28)	+0.140	+1.67	+0.00415	+0.00051

A second series of samples was taken on August 18. Sample 6 represents Acala Upland cotton growing between two rows of Pima Egyptian (sample 5). On this date it was feasible to secure samples of Pima Egyptian (7), Sea Island (8), Acala Upland (9), and Kekchi Upland (10) in close proximity in the same border. Pima Egyptian is compared with all the other types, and Sea Island is compared with the two Upland varieties. A comparison of Pima Egyptian (sample 11) with *Gossypium herbaceum* var. Wa-Gale (sample 12) from the Transcaucasian region was also made on this date.

On August 22 a third series of comparisons was made with the following samples from the yield test plots of Pima Egyptian, Lone Star Upland, and Durango Upland. In the comparisons of Pima Egyptian and Lone Star Upland cotton, samples 13-14 were taken near the south end of the border, samples 15-16 near the middle, and samples 17-18 near the north end. In the same test plot and on the same date a comparison of Pima with Durango was also made on the basis of samples near the south end (19-20), near the middle (21-22), and near the north end (23-24) of the border. In another border we took Pima Egyptian and Holdon Upland for comparison with the  $F_1$  hybrid between the two species. (Samples 25-26.) For constants for the hybrid see sample 34, p. 299. In the same border we took Pima Egyptian (27) and Acala Upland (28) for comparison with an  $F_1$  hybrid. (See No. 35, elsewhere.)

The constants for the individual samples are compared in Table I.

Comparing the values of freezing-point lowering and calculated osmotic pressure in Pima Egyptian cotton with the 13 determinations<sup>9</sup> on the several varieties of Upland cotton, we note that without exception the osmotic concentration is higher in the Egyptian cotton than in the Upland types.

Calculating the statistical constants for freezing-point lowering,  $\Delta$ , in Pima and Upland, the data presented in Table II were obtained, the mean being the average value of the constant, S. D. the standard deviation, C. V. the coefficient of variation, and  $r$ , the coefficient of correlation between the two variables denoted by the subscript.

TABLE II.—Statistical constants for freezing-point lowering in Pima and Upland cottons in 1920

	Pima, P.	Upland, U.
Mean.....	1.300±0.022	1.144±0.022
S. D.....	0.118±0.016	0.120±0.016
C. V.....	9.100	10.456

$$r_{PU} = +0.7439 \pm 0.0836$$

In determining the probable error of the difference we must remember that because of field and meteorological heterogeneity (21) the values of osmotic pressure in associated plants of Pima and Upland types may be correlated. As a matter of fact this correlation is found to be  $r = 0.744$ . This shows that the difference in salt content of the soil or differences in

<sup>9</sup> In one case (No. 7) a single sample of Egyptian cotton is compared with two varieties closely associated in the same plot of Upland cotton. In calculating the statistical constants for the whole series of each type, this one sample of Egyptian cotton is therefore used twice.

the time of the collection of the different samples influence in a somewhat similar manner the sap properties of both Pima and Upland types. The standard deviation of the difference between the constants of the two species is given by the formula

$$\sigma^2_{(P-U)} = \sigma^2_P + \sigma^2_U - 2r_{PU} \sigma_P \sigma_U.$$

Using this formula, we see that the difference in freezing point lowering of the tissue fluids of Egyptian and Upland cotton is  $0.1560 \pm 0.0159$ .

This value is 9.8 times as large as its probable error. While the number of samples is small it seems clear that, at this particular growth stage at least, the osmotic concentration of the leaf-tissue fluids of the Egyptian cotton is distinctly greater than that of the Upland. The fact that the individual differences are without exception of the same sign is a strong evidence for the validity of this conclusion.

The results for osmotic pressure,  $P$ , are stated in Table III. The difference in mean osmotic concentration is  $1.872 \pm 0.191$  atmospheres.

TABLE III.—Statistical constants for osmotic pressure in Pima and Upland cottons in 1920

	Pima, $P$ .	Upland, $U$ .
Mean.....	15.639 $\pm$ 0.265	13.767 $\pm$ 0.269
S. D.....	1.419 $\pm$ 0.188	1.437 $\pm$ 0.190
C. V.....	9.074	10.44

$$r_{PU} = +0.7436 \pm 0.0836$$

The specific electrical conductivities,  $\kappa$ , are without exception somewhat higher in Pima than in the varieties of Upland cotton with which it is compared. Calculating the statistical constants for electrical conductivity and determining the probable errors by the method above, the data presented in Table IV were obtained. The difference in the electrical conductivity is  $0.00463 \pm 0.00058$ , showing that the tissue fluids of the Egyptian cotton contain somewhat larger quantities of ionized electrolytes than do those of Upland varieties.

TABLE IV.—Statistical constants for electrical conductivity in Pima and Upland cottons in 1920

	Pima, $P$ .	Upland, $U$ .
Mean.....	0.03596 $\pm$ 0.00137	0.03133 $\pm$ 0.00119
S. D.....	0.00733 $\pm$ 0.00097	0.00633 $\pm$ 0.00084
C. V.....	20.39	20.21

$$r_{PU} = +0.9058 \pm 0.0336$$

Comparing the values of the ratio of electrical conductivity to freezing point lowering,  $\kappa/\Delta$ , for the 13 pairs of Egyptian and Upland cotton, we find that in eight cases the ratio is higher in the Egyptian than in the Upland cottons, whereas in five cases the results indicate that the relative proportion of conducting electrolytes is lower in the Egyptian than in

the Upland cottons. The differences in the ratios are relatively small. The average of the eight differences which indicate higher ratios of  $\kappa$  to  $\Delta$  in the Egyptian cotton is 0.00123, whereas the average of the five cases which indicate higher ratios of  $\kappa$  to  $\Delta$  in the Upland cotton is -0.00167. The general average for the 13 differences is  $0.00012 \pm 0.00034$ . Here the probable error has been determined by the formula given above, the statistical analysis having given the constants presented in Table V.

TABLE V.—Statistical constants for the ratio of electrical conductivity to freezing point lowering in Pima and Upland cottons in 1920

	Pima, P.	Upland, U.
Mean.....	0.02779 $\pm$ 0.00105	0.02767 $\pm$ 0.00104
S. D.....	0.00560 $\pm$ 0.00074	0.00555 $\pm$ 0.00073
C. V.....	20.17	20.04

$$r_{PV} = +0.9479 \pm 0.0189$$

Turning back to a comparison of the Egyptian with cottons other than the Upland, we note from samples 7 and 8 that the osmotic pressures and electrical conductivities are higher in Pima than in Sea Island cotton but that the ratio of electrical conductivity to freezing-point lowering is lower in Pima. Samples 11 and 12 show the same relationship to hold in a comparison of Pima Egyptian with the Transcaucasian *Gossypium herbaceum* var. Wa-Gale.

While these two cases taken alone are altogether inadequate as bases for conclusion concerning differences between Pima Egyptian cotton and other species of the genus *Gossypium*, it is to be noted that the differences are of precisely the same kind as are found in the comparison of the Egyptian and Upland types.

The results, therefore, strengthen the conclusion to be drawn from a comparison of Egyptian and Upland cottons, and suggest the interest of a more extensive comparative study of the different cotton species.

#### INVESTIGATIONS IN 1921

The organization of the experimental details of the work carried out in 1921 has been fully described above. It remains merely to state that two complete series of determinations were made and to note certain differences in the conditions of these series.

The sampling of the culture was begun on August 6, and the first series of samples—80 triplets and 60 duplets, 360 samples in all, less a few for which plants were not available—was completed on August 16.

At the time the first series was taken the soil moisture was ample but the surface layer was not wet. The plants at the beginning were in a rapidly growing condition, with but few bolls developing. Their growth was, however, being checked by the inadequacy of soil moisture when the collections were completed.<sup>10</sup>

The plants were given an ample irrigation immediately after the last samples were taken. After this irrigation there was an unusually

<sup>10</sup> Irrigation was delayed somewhat beyond the time at which water might advantageously have been applied in order that the first series of determinations might be completed in the period between two irrigations.

heavy rain. This left the fine silt in such a state that collections could not be taken up for the second series until August 19. The second series was completed on August 27.

In the first series collections were made from the single Egyptian and the single Upland plant adjoining the hybrid plant or adjoining each other. This was done in order to obtain the samples from plants subjected to as nearly identical environmental conditions as possible.

The method of collection followed in the first series has the disadvantage that the materials upon which the physical constants are to be based are drawn from single individuals, and are therefore subject to the variations characteristic of individual plants.

In taking up the second series of collections, it was evident that the two plants of Egyptian and Upland cotton from which the first samples had been taken were so depleted of the more mature leaves that it would be desirable to extend the range of individuals somewhat, and to include the adjoining plants in the taking of the samples representing the two parent forms. This could not be done in the case of the hybrid, which was represented by but a single individual in each triplet, but there was little difficulty in obtaining adequately large samples from the  $F_1$  hybrid plants, which were larger than the others.

When the second series of determinations was taken up, it seemed desirable to make all the readings in duplicate. Time was available for this extra safeguard because of the standardization of every detail of the laboratory routine which had been worked out in the first series of determinations. The conductivities were reread, frequently with a different resistance; the freezing-point determinations were repeated on the same sample of fluid, thawed after the first freezing; and the hydrogen-ion determinations were made in duplicate, or repetition readings were made with the same electrodes. The average of these duplicate determinations have been used in the final calculations of the statistical constants. Except for a very few cases in which there was an obvious slip of the pen in recording the reading, the results of the first and second determinations check remarkably well.

Limitation of space precludes the publication of the individual determinations, nearly 3,000 in number, upon which our conclusions are based. It is necessary, therefore, to summarize the determinations by treating them statistically.

This may be done in two ways: First, the determinations may be arranged in an orderly manner and the frequency distribution of the magnitudes considered; second, statistical constants may be determined for the various subseries, and conclusions based on the comparison of these statistical constants, with due regard to their probable errors.

We have first of all to consider the groupings of the materials for seriation and for the determination of statistical constants. While the constants are available for the duplets or triplets of the individual rows, it does not seem expedient for present purposes to seriate the constants by rows, or to determine the statistical constants for such a detailed grouping of the materials. We have therefore combined the rows according to variety and origin of seed.<sup>11</sup> Thus rows 1 and 5, rows 3 and 7, and rows 2, 4, and 6 have been combined.

<sup>11</sup> In working with the original records for another purpose we have been careful to note that the conclusions drawn for the combined materials of the individual varieties are in general substantiated by the results for the individual rows. Because of the influence of the number of determinations on the probable errors of random sampling, the constants for the individual rows will show greater irregularity than those based on the combined records of two or more rows.

These represent the fundamental units on which the conclusions are based. Since rows 1 and 5 and 3 and 7 do not differ in variety but only in the origin of the seed used, the four rows (1, 3, 5, and 7) have been combined for a more extensive comparison of Pima and Meade cotton.

This has given a series of semi-independent subsamples. The concordance of the results from these subsamples is one of the strongest arguments for the validity of the conclusions based on the series as a whole.

In comparing the differences between the parental types, or between the parental types and the hybrids, we must remember that the two or three determinations on the same duplet or triplet will be rendered more or less alike by (a) the heterogeneity of the plot upon which the plants were grown and by (b) the differences in the time at which the samples were taken. Thus we should expect a correlation between the constants of the individuals of the same duplet or triplet, notwithstanding the fact that these individuals belong to different varieties of cotton and are merely rendered similar by community of environmental influences. The consequence of these correlations for the arguments of the present section of the investigation is that the probable error of the differences between the two parental types, and between the two individual parental types and the hybrid form, will be greatly reduced. We have, therefore, always calculated the probable error of the difference between the series of constants with due regard to the intensity of correlation between them.

In the analysis of the data we may first present the frequency distributions for the physicochemical measurements made. We shall then proceed to a more detailed comparison of the physicochemical constants by means of statistical constants.

We may now consider the frequency distributions of the constants for the individual classes of plants. The depression of the freezing point may be expressed in units of 5 per cent of the molecular lowering taken as  $\Delta = 1.86$ . The results are given in Table VI.

Inspection of these distributions shows clearly that the constants for Pima are somewhat higher than those for Meade. The numbers are rather too small to indicate clearly the nature of the distribution. In some cases they are wholly skew, but in general there is a suggestion of a more or less symmetrical distribution about a central mode. The frequencies for the hybrid suggest a lower minimum than that found in either of the parent forms.

The distribution of the measures of specific electrical conductivity is shown in Table VII. The distribution of the ratios of specific electrical conductivity to the freezing point lowering is represented in Table VIII.

The results for specific electrical conductivity show that the Egyptian cotton has a somewhat higher maximum than that found in the comparable Upland series. It is difficult to decide merely from inspection of the distributions the relative value of the ratio  $\kappa/\Delta$  in the Upland, in the Egyptian, and in the  $F_1$  forms.

The frequencies for the hydrogen-ion concentrations are shown in Table IX. Apparently the values of  $P_H$  are somewhat higher in the Upland than in the Egyptian form. The relation of the hybrid to the two parental forms can not be determined by inspection merely.

TABLE VI.—Distribution of magnitudes of depression of freeing point,  $\Delta$ , in the various comparisons of Pima Egyptian, Meade and Acala Upland, and  $F_1$  hybrid cotton in the first and second series of determinations in 1921

Type and variety of cotton and nature of seed planted.	Rows.	Series or collections.	0.884 to 0.976.	0.977 to 1.069.	1.070 to 1.162.	1.163 to 1.255.	1.256 to 1.348.	1.349 to 1.441.	1.442 to 1.534.	1.535 to 1.627.	1.628 to 1.720.	1.721 to 1.813.	1.814 to 1.906.	Total.
Pima Egyptian, from self-fertilized seed.	1,5	First (1)	.....	.....	.....	11	8	3	9	3	4	.....	1	39
Meade Upland, from self-fertilized seed.	1,5	First (1)	.....	.....	1	10	10	7	7	0	.....	.....	.....	39
$F_1$ hybrid, from self-fertilized seed.	1,5	First (1)	.....	.....	7	7	12	6	3	4	.....	.....	.....	39
Pima Egyptian, from self-fertilized seed.	1,5	Second (2)	.....	.....	3	12	13	5	5	1	.....	.....	.....	39
Meade Upland, from self-fertilized seed.	1,5	Second (2)	.....	.....	15	12	5	7	.....	.....	.....	.....	.....	39
$F_1$ hybrid, from self-fertilized seed.	1,5	Second (2)	.....	.....	16	6	6	1	.....	.....	.....	.....	.....	39
Pima Egyptian, from bulk seed.	3,7	First (1)	.....	.....	1	4	15	9	8	1	.....	.....	.....	38
Meade Upland, from bulk seed.	3,7	First (1)	.....	.....	2	7	8	11	10	.....	.....	.....	.....	38
$F_1$ hybrid, from bulk seed.	3,7	First (1)	.....	.....	3	8	13	7	2	.....	.....	.....	.....	38
Pima Egyptian, from bulk seed.	3,7	Second (2)	.....	.....	6	13	14	4	1	.....	.....	.....	.....	38
Meade Upland, from bulk seed.	3,7	Second (2)	.....	.....	15	16	6	1	.....	.....	.....	.....	.....	38
$F_1$ hybrid, from bulk seed.	3,7	Second (2)	.....	.....	12	7	3	.....	.....	.....	.....	.....	.....	38
Pima Egyptian, combined series.	1,3,5,7	First (1)	.....	.....	1	15	23	.....	.....	.....	.....	.....	.....	77
Meade Upland, combined series.	1,3,5,7	First (1)	.....	.....	3	17	18	13	17	4	4	.....	.....	77
$F_1$ hybrid, combined series.	1,3,5,7	First (1)	.....	.....	10	15	30	13	10	5	.....	.....	.....	77
Pima Egyptian, combined series.	1,3,5,7	Second (2)	.....	.....	9	25	27	9	6	1	.....	.....	.....	77
Meade Upland, combined series.	1,3,5,7	Second (2)	.....	.....	30	28	11	8	.....	.....	.....	.....	.....	77
$F_1$ hybrid, combined series.	1,3,5,7	Second (2)	.....	.....	28	13	9	1	.....	.....	.....	.....	.....	77
Pima Egyptian, from bulk seed.	2,4,6	First (1)	.....	.....	3	13	20	13	0	.....	4	.....	.....	59
Meade Upland, from bulk seed.	2,4,6	First (1)	.....	.....	5	21	14	13	.....	.....	.....	.....	.....	59
$F_1$ hybrid, from bulk seed.	2,4,6	First (1)	.....	.....	5	22	15	17	.....	.....	1	.....	.....	59
Pima Egyptian, from bulk seed.	2,4,6	Second (2)	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	59
Acala Upland, from bulk seed.	2,4,6	Second (2)	.....	.....	7	18	11	3	.....	.....	.....	.....	.....	59





TABLE VIII.—Distribution of magnitudes of the ratio of specific electrical conductivity to the freezing point lowering,  $\kappa/\Delta \times 10^6$ , in the various comparisons of *Pima Egyptian*, *Meade* and *Acala Upland*, and *F<sub>1</sub>* hybrid cotton, in 1921, in the first and second series of determinations

Type and variety of cotton and nature of seed planted.	Rows.	Series or col- lections.	1750 to 1850.	1850 to 1950.	1950 to 2050.	2050 to 2150.	2150 to 2250.	2250 to 2350.	2350 to 2450.	2450 to 2550.	2550 to 2650.	2650 to 2750.	2750 to 2850.	2850 to 2950.	Total.
<i>Pima Egyptian</i> , from self-fertilized seed.	1, 5	First (1)				3	5	10	7	4	9	1			39
<i>Meade Upland</i> , from self-fertilized seed.	1, 5	First (1)				1	5	12	9	6	6				39
<i>F<sub>1</sub></i> hybrid.	1, 5	First (1)		I		2	5	12	11	2	1	2		I	39
<i>Pima Egyptian</i> , from self-fertilized seed.	1, 5	Second (2)			I	4	5	4	16	5	2		2		39
<i>Meade Upland</i> , from self-fertilized seed.	1, 5	Second (2)			I	3	7	12	9	4	2				39
<i>F<sub>1</sub></i> hybrid.	1, 5	Second (2)					2	3	12	9	5	3			39
<i>Pima Egyptian</i> , from bulk seed.	3, 7	First (1)				1	9	10	5	8	3				38
<i>Meade Upland</i> , from bulk seed.	3, 7	First (1)		2		1	4	4	8	9	1				38
<i>F<sub>1</sub></i> hybrid.	3, 7	First (1)		I			6	9	13	4	4				38
<i>Pima Egyptian</i> , from bulk seed.	3, 7	Second (2)					7	9	14	5	1				38
<i>Meade Upland</i> , from bulk seed.	3, 7	Second (2)	I			4	1	15	10	1					38
<i>F<sub>1</sub></i> hybrid.	3, 7	Second (2)			2		1	7	7	13					38
<i>Pima Egyptian</i> , combined series.	1, 3, 5, 7	First (1)		I		4	14	20	12	13	7				77
<i>Meade Upland</i> , combined series.	1, 3, 5, 7	First (1)			2	4	13	16	17	15	7				77
<i>F<sub>1</sub></i> hybrid, combined series.	1, 3, 5, 7	First (1)		2		5	11	21	24	6	5	2		I	77
<i>Pima Egyptian</i> , combined series.	1, 3, 5, 7	Second (2)				5	12	13	30	10	3		2		77
<i>Meade Upland</i> , combined series.	1, 3, 5, 7	Second (2)			I	7	8	27	19	9	2				77
<i>F<sub>1</sub></i> hybrid, combined series.	1, 3, 5, 7	Second (2)	I		3		7	10	17	27	12				77
<i>Pima Egyptian</i> , from bulk seed.	2, 4, 6	First (1)		I		1	3	16	17	9	6				59
<i>Meade Upland</i> , from bulk seed.	2, 4, 6	First (1)			I	1	6	17	17	8	13				59
<i>F<sub>1</sub></i> hybrid, from bulk seed.	2, 4, 6	First (1)				2	1	23	14	7	3			I	59
<i>Pima Egyptian</i> , from bulk seed.	2, 4, 6	Second (2)				1	9	25	14	7	3				59
<i>Meade Upland</i> , from bulk seed.	2, 4, 6	Second (2)				1	9	25	14	7	3				59



These frequency distributions show two things:

The values of all the physicochemical properties investigated show considerable variability from sample to sample.

There are evidences of differences in the constants for the two parent forms and for the  $F_1$  hybrid. It is clear, however, that for an analysis of these differences we must turn to a more careful study of statistical constants. Since, however, the utmost precision is required we have determined the constants directly from the ungrouped data. These have been recorded to four significant figures in the case of  $\Delta$ , to five significant figures in the case of  $\kappa$  and  $\kappa/\Delta$ , and to three significant figures in the case of  $P_H$ . These values are of course beyond the range of precision of the instruments employed, but they represent as close an approximation as it was possible to secure in the case of a single reading, and in the second series the results are, in practically all cases, the average of two readings.

#### COMPARISON ON THE BASIS OF FREEZING POINT DEPRESSIONS

The comparison between the various series of Pima Egyptian and Meade and Acala Upland cottons is made in Table X. Since all of the tables in which comparisons are drawn between the physicochemical properties of the Egyptian and Upland varieties are constructed in the same way, one explanation will suffice for those for  $\kappa$ ,  $\kappa/\Delta$  and  $P_H$  (Tables XI to XIII), as well as for  $\Delta$ .

The varieties compared are given in column 1. For each variety two, and sometimes three, rows of plants are available.<sup>12</sup> By combining the Pima and Meade grown from self-fertilized and bulk seed respectively four rows are available for a comparison of Pima and Meade. In column 2 are given the numbers of the rows from which the samples were taken. Two sets of constants are available for each variety. The upper entry represents in each case the constants derived from the first series of determinations (August 6 to 16) while the lower entry represents the constants derived from the second series of determinations (August 19 to 27). Column 3 gives the number of individual determinations upon which the statistical constants are based. Column 4 indicates the series (i. e., the first or second collection) and the method of taking the differences in drawing the comparison between the constants for the earlier and for the later period. Column 5 gives the average value of freezing-point depression for Egyptian cotton; column 6 gives the comparable value for Upland cotton. Column 7 gives the correlation coefficient measuring the relationship between the constants of the plants, or small groups of plants, grown in immediate association in the various duplets or triplets. The purpose of these coefficients will be discussed presently.

Two sets of differences appear.

The first set, entered opposite the row numbers in columns 8-10, show the differences between Egyptian and Upland cotton. The differences in column 8 are so taken that a positive sign indicates a greater freezing point depression in the Egyptian than in the Upland cotton. These constants are provided with probable errors, calculated by the equation given above (p. 279). Column 9 shows the ratio of these differences to their probable errors.<sup>13</sup> The differences as given in column 8 are absolute

<sup>12</sup> The location of these rows in the field will be clear from the map, fig. 1, p. 272.

<sup>13</sup> These ratios have been calculated from the values of the differences and their probable errors carried to a larger number of places than can be given in the tables of this paper. They are, therefore, somewhat more accurate than those which may be recomputed from the values of the constants as given here.

TABLE X.—Comparison of freezing point depression,  $\Delta$ , in Pima Egyptian and Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921

(1) Types and varieties compared.	(2) Rows.	(3) N	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland.		
							(8) Absolute difference.	(9) Diff. — Eaff.	(10) Per- cent- age differ- ence.
Pima Egyptian and Meade Upland, from self-fertilized seed.....	{ 1, 5 1, 5	39 39	First (1).....	1.4037 $\pm$ . 0179	1.3528 $\pm$ . 0137	+ .8583	+ .0599 $\pm$ . 0093	5.46	3.76
			Second (2).....	1.2983 $\pm$ . 0113	1.2122 $\pm$ . 0107	+ .7654	+ .0861 $\pm$ . 0070	11.4	7.10
			Diff. (2) — (1).....	6.39	8.06	— .0929	+ .0352 $\pm$ . 0109	.....	.....
Pima Egyptian and Meade Upland, from bulk seed.....	{ 3, 7 3, 7	38 38	Diff./Eaff.....	.....	.....	.....	3.21	.....	.....
			First (1).....	1.3572 $\pm$ . 0110	1.3515 $\pm$ . 0125	+ .6683	+ .0057 $\pm$ . 0096	0.59	0.42
			Second (2).....	1.2573 $\pm$ . 0093	1.2010 $\pm$ . 0082	+ .6616	+ .0563 $\pm$ . 0072	7.77	4.69
Pima Egyptian and Meade Upland, combined series.....	{ 1, 3, 5, 7 1, 3, 5, 7	77 77	Diff. (2) — (1).....	8.21	10.7	— .0067	+ .0506 $\pm$ . 0122	.....	.....
			Diff./Eaff.....	.....	.....	.....	4.15	.....	.....
			First (1).....	1.3808 $\pm$ . 0107	1.3522 $\pm$ . 0093	+ .7687	+ .0286 $\pm$ . 0069	4.13	2.12
Pima Egyptian and Acala Upland, from bulk seed.....	{ 2, 4, 6 2, 4, 6	59 59	Second (2).....	1.2780 $\pm$ . 0075	1.2667 $\pm$ . 0068	+ .7222	+ .0713 $\pm$ . 0054	13.3	5.91
			Diff. (2) — (1).....	9.98	12.9	— .0465	+ .0427 $\pm$ . 0082	.....	.....
			Diff./Eaff.....	.....	.....	.....	5.22	.....	.....
Pima Egyptian and Acala Upland, from bulk seed.....	{ 2, 4, 6 2, 4, 6	59 59	First (1).....	1.3401 $\pm$ . 0112	1.2998 $\pm$ . 0106	+ .6795	+ .0403 $\pm$ . 0088	4.60	3.10
			Second (2).....	1.2804 $\pm$ . 0076	1.1822 $\pm$ . 0086	+ .6441	+ .0982 $\pm$ . 0069	14.2	8.31
			Diff. (2) — (1).....	6.00	8.49	— .0354	+ .0579 $\pm$ . 0095	.....	.....
			Diff./Eaff.....	.....	.....	.....	6.11	.....	.....

values. For purposes of comparison it is desirable to reduce these to a percentage basis by dividing all of the absolute differences ( $\times 100$ ) by the values for the Upland cotton.<sup>14</sup> The resulting percentage differences appear in column 10.

The second set of differences—those entered under the average constants for the first and second collections of Egyptian and Upland cottons, respectively, in columns 5 and 6, under the first and second correlation coefficients in column 7, and under the differences between Egyptian and Upland cotton in the first and second series in column 8—shows the relative values of the constants secured in the first and in the second series of determinations.

All of these differences are taken—

(constant for second series) *minus* (constant for first series).

All of the differences have been taken algebraically, with regard to the signs of the constants compared.

Since the physical constants for the two types of cotton (columns 5 and 6) are both positive values, a positive sign indicates a higher value of the physical constant in the second series. Since the correlations (column 7) and the differences between the two types of cotton (column 8) may theoretically be either positive or negative (and actually differ with regard to sign in some of the subsequent tables), it is necessary in interpreting these differences to bear in mind the signs of the constants for the first and second series between which comparisons are being drawn.

The ratios of these differences to their probable errors (when probable errors have been determined) are shown below the differences (opposite  $\text{Diff.}/E_{\text{diff.}}$ ).<sup>15</sup>

In determining the probable errors of differences between the means for the two series (columns 5 and 6) the correlation between the constants of the first and second series of determinations must be considered. The standard deviations of the differences are calculated from the formula

$$\sigma \frac{2}{(2-1)} = \sigma_1^2 + \sigma_2^2 - 2r_{12} \sigma_1 \sigma_2,$$

where the sigmas denote standard deviations and  $r$  the correlation coefficient measuring the relationship between the first and second series, as designated by the subscript numerals.<sup>16</sup>

The differences between the differences between Egyptian and Upland cotton in the first and second series (column 8, opposite "Diff., (2)-(1)") show whether the two types show greater or less differentiation at the time of the second than at the time of the first collection of samples.

The probable errors of the increase in the differences between the two kinds of cotton have been calculated as follows:

Let E. U. denote Egyptian and Upland plants, of the first or second series as may be indicated by subscript numerals. Then the differences are  $E_1 - U_1$  and  $E_2 - U_2$ . The numerical value under consideration in

<sup>14</sup> In the case of hydrogen-ion concentration (Table XIII) the value of the deviation of the  $P_a$  for the Upland cotton from neutrality has been used in the calculation of the percentage difference between the two types.

<sup>15</sup> These values, like the ratios mentioned immediately above, have been computed from the original constants carried to a larger number of places than those given here, and are consequently slightly more accurate than those which may be recomputed from the constants as given in these tables.

<sup>16</sup> The coefficients of correlation,  $r_{12}$ , are given for each of the physicochemical constants considered, and for the different subcultures, in Table XXI. These coefficients will be considered later (p. 313). For the moment they are of interest merely as a means to the calculation of the probable errors of the differences between the constants for the first and second series of determinations.

the comparison of the first and second series is  $(E_2 - U_2) - (E_1 - U_1)$ . The means, standard deviations and correlations of these differences might be determined directly. Since the means and standard deviations of the individual determinations are both available and since the correlations between the individual determinations in the first and second series may be utilized for another investigation, it seems best to determine the correlations between the differences in the first series and the second series indirectly. The type of formula to be applied has been given elsewhere (22) and illustrated in its applicability to growth increments (31).

The formula to be used in the calculation of the probable error of the difference between the differences has also been given (23).

The standard deviations of the difference between the two parental forms have been determined in order to test the significance of the difference between the types in either the first or second series. We require, therefore, merely the correlations between the differences in the individual duplets or triplets, in the first series and in the second series.

In this case the product moment for the correlation between the differences,  $d_1$ ,  $d_2$

$$\Sigma (d_1 d_2) = \Sigma (E_1 E_2) - \Sigma (E_1 U_2) - \Sigma (U_1 E_2) + \Sigma (U_1 U_2).$$

The standard deviation of the differences between the differences in the first and second series then becomes—

$$\sigma^2_{(d_2 - d_1)} = \sigma_{d_1}^2 + \sigma_{d_2}^2 - 2r_{d_1 d_2} \sigma_{d_1} \sigma_{d_2}.$$

The correlations between these differences for the various characters appear in Table XXIII.

Turning now to a consideration of the actual constants in Table X we note first of all that the differences between Egyptian and Upland cotton (column 8) are without exception positive in sign. Thus they show that in all instances the leaf-tissue fluids of the Egyptian cotton have a higher osmotic concentration than those of the Upland. The differences in the first series are, in all cases but one, at least four times as large as their probable errors (column 9). The single exception occurs in the comparison between bulk Pima and bulk Meade. In the second series the differences are from 7.8 to 14.2 times as large as their probable errors. There can, therefore, be no question whatsoever concerning the significance of the differences between the osmotic concentration of the tissue fluids of Egyptian and Upland cotton. Expressing the results in terms of differences in atmospheres,  $P$ , rather than in freezing point lowering,  $\Delta$ , by means of the formula

$$P = 12.06 \Delta - 0.021 \Delta^2,$$

for which tabled constants are available (24), it appears that the differences range from about 0.07 to 0.62 atmospheres in the first series. In the second series the differences range from 0.68 to 1.18 atmospheres.

The differences between the first and second series of determinations (Table X, columns 5 and 6) show that in the second series the osmotic concentration is regularly lower than that in the first series. These differences are in all cases six or more times as large as their probable errors. They show conclusively, therefore, that osmotic concentration is lower in the second series of determinations than in the first.

The correlations between members of the same duplet or triplet (column 7) are in all cases higher in the first than in the second series.

Since the problem of the magnitudes of the correlation between the members of the same duplet or triplet is not a subject for special discussion in the present analysis of the measurements, the probable errors of the differences between these correlation coefficients for the first and second series of determination have not been determined.

Finally, we note that the differences between Egyptian and Upland cotton are uniformly higher in the second than in the first series of collections. This is clearly shown by the absolute differences in column 8. The increase in the absolute difference between the Egyptian and Upland cotton from the first to the second series of determinations may be shown by taking the differences between these differences. These are shown immediately beneath the two differences.

The increase in the difference between Egyptian and Upland from the first to the second series of determinations is in all cases over three times as large as its probable error. In three cases it is over four times as large as its probable error.

Correlated with this larger difference we find a uniformly higher criterion of significance of difference (column 9) between the Egyptian and the Upland types in the second series.

The constants just discussed represent the absolute differences between the Egyptian and the Upland cottons as studied in the first and second series of determinations. It must be remembered, however, that in both Egyptian and Upland types the osmotic concentration is uniformly lower in the second series than in the first series of determinations. This means that the differences which have been shown to be absolutely larger in the second series than in the first are relatively much larger. This is clearly shown by the entries in the column (10) of Table X giving the percentage differences between the two types. In the first series of determinations, the relative differences range from less than 1 per cent to over 3.7 per cent. In the second series the percentage differences range from 4.7 per cent to 8.3 per cent.

The significance of these various comparisons will be more fully discussed in a subsequent section after the values of the other physical constants have been taken into account.

#### COMPARISON ON THE BASIS OF SPECIFIC ELECTRICAL CONDUCTIVITY

The statistical constants for the specific electrical conductivity of the tissue fluids of Egyptian and Upland cottons, expressed in terms of reciprocal ohms,  $\kappa \times 10^6$ , are compared in Table XI, which is made up in the same manner as Table X.

The results show that in every comparison (column 8) the tissue fluids of the Egyptian cotton have a higher specific electrical conductivity than those of the Upland varieties with which they are compared. A comparison of the differences with their probable errors (which have been calculated with due regard to the correlation obtaining between the constants of plants of the same duplet or triplet), shows (column 9) that in the first series all of the differences are over three times as large as their probable errors. In the second series all of the differences in electrical conductivity are over nine times as large as their probable errors.

TABLE XI.—Comparison of specific electrical conductivity,  $\kappa \times 10^6$ , in Pima Egyptian and Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian Upland.	Difference between Egyptian and Upland.		
							(8) Absolute difference.	(9) Diff. $E_{\text{uff}}$ .	(10) Percentage difference.
Pima Egyptian and Meade Upland, from self-fertilized seed.	{ 1, 5 1, 5	39 39	First (1).....	33, 251 $\pm$ 300	32, 041 $\pm$ 261	+ .6753	+1, 210 $\pm$ 229	5.29	3.77
			Second (2).....	30, 643 $\pm$ 237	28, 056 $\pm$ 222	+ .3644	+2, 587 $\pm$ 259	9.99	9.22
Pima Egyptian and Meade Upland, from bulk seed.	{ 3, 7 3, 7	38 38	Diff. (2)-(1).. Diff./ $E_{\text{uff}}$ .....	-2, 608 $\pm$ 331 7.87	-3, 985 $\pm$ 356 11.2	- .3109	+1, 377 $\pm$ 314 4.38		
			First (1)..... Second (2).....	32, 074 $\pm$ 201 29, 646 $\pm$ 202	31, 141 $\pm$ 253 27, 402 $\pm$ 186	+ .5706 + .2844	+933 $\pm$ 251 +2, 244 $\pm$ 233	3.71 9.65	2.99 8.19
Pima Egyptian and Meade Upland, combined series.	{ 1, 3, 5, 7 1, 3, 5, 7	77 77	Diff. (2)-(1).. Diff./ $E_{\text{uff}}$ .....	-2, 428 $\pm$ 339 7.15	-3, 739 $\pm$ 317 11.8	- .2952	+1, 311 $\pm$ 371 3.53		
			First (1)..... Second (2).....	32, 671 $\pm$ 216 30, 151 $\pm$ 161	31, 597 $\pm$ 185 27, 733 $\pm$ 147	+ .6378 + .3584	+1, 074 $\pm$ 173 +2, 418 $\pm$ 175	6.21 13.83	3.40 8.72
Pima Egyptian and Acala Upland, from bulk seed.	{ 2, 4, 6 2, 4, 6	59 59	Diff. (2)-(1).. Diff./ $E_{\text{uff}}$ .....	-2, 520 $\pm$ 230 11.0	-3, 864 $\pm$ 239 16.2	- .2794	+1, 344 $\pm$ 235 5.72		
			First (1)..... Second (2).....	32, 028 $\pm$ 216 29, 981 $\pm$ 169	31, 117 $\pm$ 181 27, 606 $\pm$ 158	+ .4881 + .4168	+911 $\pm$ 204 +2, 375 $\pm$ 177	4.47 13.42	2.93 8.60
			Diff. (2)-(1).. Diff./ $E_{\text{uff}}$ .....	-2, 047 $\pm$ 235 8.70	-3, 511 $\pm$ 256 13.7	- .0653	+1, 464 $\pm$ 275 5.32		



Thus it is clear that the Egyptian cotton contains larger quantities of conducting electrolytes in its leaf tissue fluids, just as it has been shown in the preceding section to contain larger quantities of osmotically active solutes.

Comparisons between the first and the second series show that in both Egyptian and Upland cotton (Table XI, columns 5 and 6) the electrical conductivity in the second series is invariably lower than in the first series. The differences are in all cases over 7.2 times as large as their probable errors and may be considered statistically significant in every instance.

The correlation between the plants of the same duplet or triplet is smaller in the second series than in the first. This result is in full agreement with that for osmotic concentration presented above. Since these correlations are not discussed in detail in this place, but merely served as a means of determining certain probable errors which are essential to the interpretation of the other constants, their probable errors, and the probable errors of the differences between them, have not been determined.

The differences between the two types of cotton (Table XI, column 8) are in all cases greater in the second than in the first series. The differences between Egyptian and Upland cotton in reciprocal ohms are over twice as great in the second as in the first series.

The ratio of these differences between the differences to their probable errors (calculated with due regard to the correlation between the differences themselves as explained above and shown in column 8) varies from 3.5 to 5.7 and may be reasonably considered to indicate statistically significant differences.

The ratios of the differences between the Egyptian and the Upland plants to their probable errors (column 9) are regularly larger in the second than in the first series of determinations.

Since the differences are based upon smaller absolute values in the second series than in the first, they are relatively larger than they appear to be as shown in column 8. The percentage differences in column 10 show that in the first series of determinations the Egyptian cotton has from 2.9 to 3.8 per cent higher electrical conductivity than the associated Upland cotton. In the second series of determinations, the Egyptian cotton is characterized by from 8.2 to 9.2 per cent higher electrical conductivity of its leaf tissue fluids.

COMPARISON ON THE BASIS OF THE RATIO OF SPECIFIC ELECTRICAL CONDUCTIVITY TO FREEZING POINT DEPRESSION

We now have to consider the relative proportion of electrolytes as indicated by the ratio of the specific electrical conductivity to freezing point lowering,  $\kappa/\Delta$ .

The comparisons between the Upland and Egyptian varieties appear in Table XII which is constructed on the same principle as Table X. For the first series the differences between the Egyptian and the Upland types (column 8) are in three cases positive and one case negative in sign. In none of these four cases is the difference as much as two and one-half times as large as its probable error. We must, therefore, conclude that the two forms have practically the same average ratios.

TABLE XII.—Comparison of ratio of specific electrical conductivity to freezing-point depression,  $\kappa/\Delta_{10}^0$ , in *Pima Egyptian* and *Meade and Acala Upland* cotton, and in the first and second series of determinations for each type, in 1921

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland.		
							(8) Absolute Difference.	(9) Diff. E. diff.	(10) Percentage difference.
<i>Pima Egyptian</i> and <i>Meade Upland</i> from self-fertilized seed.	{ 1, 5 1, 5	39 39	First (1).....	23812 ± 172	23753 ± 141	+ .4310	+ 59 ± 109	0.35	0.25
			Second (2).....	23669 ± 173	23195 ± 151	+ .1109	+ 474 ± 217	2.18	2.04
<i>Pima Egyptian</i> and <i>Meade Upland</i> , from bulk seed.	{ 3, 7 3, 7	38 38	Diff. (2) - (1) ..	-143 ± 194	-558 ± 200	- .3201	+ 415 ± 258		
			Diff./E <sub>upl.</sub> .....	0.74	2.79		1.61		
			First (1).....	23954 ± 160	23139 ± 213	+ .4050	+ 515 ± 208	2.47	2.23
			Second (2).....	23017 ± 141	22864 ± 108	+ .5099	+ 753 ± 145	5.19	3.29
<i>Pima Egyptian</i> and <i>Meade Upland</i> , combined series.	{ 1, 3, 5, 7 1, 3, 5, 7	77 77	Diff. (2) - (1) ..	-37 ± 171	-275 ± 284	+ .1049	+ 238 ± 245		
			Diff./E <sub>upl.</sub> .....	0.21	0.97		0.97		
			First (1).....	23734 ± 118	23450 ± 129	+ .4064	+ 284 ± 135	2.10	1.21
			Second (2).....	23043 ± 112	23032 ± 114	+ .3189	+ 611 ± 132	4.65	2.65
<i>Pima Egyptian</i> and <i>Acala Upland</i> , from bulk seed.	{ 2, 4, 6 2, 4, 6	59 59	Diff. (2) - (1) ..	-91 ± 130	-418 ± 173	- .0805	+ 327 ± 184		
			Diff./E <sub>upl.</sub> .....	0.70	2.42		1.78		
			First (1).....	23969 ± 119	24044 ± 152	+ .3826	- 76 ± 153	0.50	0.37
			Second (2).....	23451 ± 113	23399 ± 93	+ .4159	+ 52 ± 113	0.40	0.22
			Diff. (2) - (1) ..	-517 ± 145	-645 ± 168	+ .0333	+ 127 ± 168		
			Diff./E <sub>upl.</sub> .....	8.56	3.85		0.70		

In the second series the signs of all four of the differences (column 8) indicate a slightly higher ratio,  $\kappa/\Delta$ , in the Egyptian plants. One of the differences is certainly insignificant in comparison with its probable error. The others may or may not be significant.

Finally, the percentage column (10) shows that the differences between Upland and Egyptian cotton do not exceed 3.3 per cent of the value of the constant for the Upland form.

The net result of this comparison seems to be that, taking the materials as a whole, there is no certain evidence of a difference in the relative values of  $\kappa$  in the two groups of cottons. Such differences as do appear are not wholly consistent from series to series and may be due merely to the errors of random sampling or to heterogeneity in the field upon which the plants were grown. The results for both the first and second series of determinations suggest, however, that the ratio is slightly higher in Egyptian than in Upland cotton. It will not be worth while to consider this problem further until conductivities can be corrected for the viscosity of the fluid, or until comparisons can be based on the actual analytical determination of one or more of the electrolyte ions.

A comparison of the ratio  $\kappa/\Delta$  in the first and second series of determinations (Table XII) shows that in both Egyptian (column 5) and Upland (column 6) cotton, the ratio is somewhat smaller in the second than in the first series. These differences are not sufficiently large in comparison with their probable errors to be considered definitely significant as individual constants. The consistency of the results evidences strongly for a relatively lower concentration of electrolytes in the determinations made later in the season.

The differences between Egyptian and Upland cotton are apparently larger in the second than in the first series.

#### COMPARISON ON THE BASIS OF HYDROGEN-ION CONCENTRATION

While various authors have determined the total acidity of plant tissue fluids by titration, we have relatively little information on their actual acidity as determined by the systematic use of indicators, and fewer still by electrometric methods, giving the true hydrogen-ion concentration. Much of the work has been of a wholly incidental nature, and no attempt will be made here to review it in detail. Reference may be made to a recent summary by Atkins (3).

Haas (18, 19), Truog (50), Hoagland (34), and Atkins (1) have shown that, in general, plant saps have a hydrogen-ion concentration well on the acid side of neutrality. Our findings for cotton are in agreement with this general experience, although the acidities which we have found are not so high as those stated by Atkins (1).

As far as we are aware, detailed studies of the relative values of hydrogen ion concentration in agricultural varieties have not been made heretofore.

The constants for the acidity of the tissue fluids of the Egyptian and Upland cottons measured in terms of  $P_H$  appear in Table XIII.

The average values of  $P_H$  in the various series of Egyptian cotton (column 5) range from 5.24 to 5.41, while in the Upland cotton (column 6) they range from 5.30 to 5.46. Thus all averages are well on the acid side of neutrality.

Comparing the Upland and Egyptian cotton by means of the difference column (8), we note that the  $P_H$  values for Egyptian cotton are in all cases lower than those for the Upland cottons.

The ratios of the differences to their probable errors (column 9) are in all cases larger than, and in some cases several times as large as, 3.5 times their probable error. There can, therefore, be no reasonable question concerning their significance. The results show clearly that the tissue fluids of the Egyptian cotton are distinctly more acid than those of the Upland cottons.

In the calculation of the percentage difference between the hydrogen-ion concentrations of the tissue fluids of the two species, a procedure must be adopted which is somewhat different from that used for the other constants. When expressed in terms of  $P_H$ , both acidity and alkalinity are measured from a neutral point determined by the dissociation constant of pure water. We may, therefore, logically take the deviation of the  $P_H$  value of the Upland cotton from neutrality as a base in calculating the percentage differences.

The exact value of  $P_H$  taken to represent neutrality is of relatively little importance. The dissociation constant of water varies with the temperature. We may arbitrarily take  $P_H = 6.860$  for neutrality at the average temperature of our determinations. In determining the percentage difference between Egyptian and Upland cotton we therefore work from the formula

$$\frac{100 (E - U)}{6.860 - U}$$

where  $E$  and  $U$  represent the  $P_H$  values of Egyptian and Upland cottons, respectively.

The percentage differences (Table XIII, column 10) calculated in this way range from 2.75 to 8.67.

The correlations between the Upland and Egyptian plants of the same duplet or triplet (Table XIII, column 7) are of considerable interest. The values for the second series are very much larger than those for the first. All of the coefficients are positive in sign. These results are of importance in that they show that the hydrogen-ion concentrations of adjoining plants are correlated. This must be assumed to be due either to (a) extremely local differences in the soil of the field, or to (b) the influence of variations in the atmospheric conditions or in the time of collection of the samples. It is impossible at present to make any suggestions concerning the real factor to which these correlations are due. Their existence indicates clearly, however, that here is an important field for further investigation.

Turning now to the differences between the first and second collections (Table XIII, columns 5 and 6), we note that in all cases the value of  $P_H$  is somewhat larger in the second series than in the first. Thus the tissue fluids were on the average more nearly neutral at the time of the second series of determinations than when the first series was made.

A comparison of these differences with their probable errors shows that in every comparison except that based on plants grown from bulk seed of Meade in rows 3 and 7 the differences are at least 6 times as large as their probable errors.

There can, therefore, be no reasonable doubt concerning the validity of the conclusion that at the time of the taking of the second series of samples the tissue fluids of both Egyptian and Upland cultures were more nearly neutral than they were earlier in the season.

TABLE XIII.—Comparison of hydrogen-ion concentration,  $V_H$ , in Pima Egyptian, Meade and Acala Upland cotton, and in the first and second series of determinations for each type, in 1921

(1) Types and varieties compared.	(2) Rows.	(3) N.	(4) Series or collection.	(5) Mean for Egyptian cotton.	(6) Mean for Upland cotton.	(7) Correlation between Egyptian and Upland.	Difference between Egyptian and Upland.		
							(8) Absolute difference.	(9) Diff. E. diff.	(10) Percentage difference.
Pima Egyptian and Meade Upland, from self-fertilized seed.	{ 1, 5 1, 5	39 39	First (1).....	5.245 ± .012	5.346 ± .012	+ .4045	-.101 ± .013	7.84	6.67
			Second (2).....	5.335 ± .008	5.435 ± .009	+ .5580	-.120 ± .008	14.7	8.54
Pima Egyptian and Meade Upland, from bulk seed.	{ 3, 7 3, 7	38 38	Diff. (2)-(1).....	+ .090 ± .014	+ .109 ± .014	+ .1535	-.019 ± .015		
			Diff. $V_H$ .....	6.26	7.90		1.27		
			First (1).....	5.260 ± .011	5.306 ± .012	+ .1334	-.127 ± .015	8.48	8.67
			Second (2).....	5.378 ± .012	5.430 ± .012	+ .6154	-.058 ± .010	5.54	4.07
Pima Egyptian and Meade Upland, combined series.	{ 1, 3, 5, 7 1, 3, 5, 7	77 77	Diff. (2)-(1).....	+ .109 ± .015	+ .040 ± .018	+ .4820	+ .060 ± .019		
			Diff. $V_H$ .....	7.12	2.23		3.61		
			First (1).....	5.257 ± .008	5.370 ± .009	+ .2949	-.113 ± .010	11.5	7.58
			Second (2).....	5.357 ± .007	5.446 ± .007	+ .5530	-.089 ± .007	12.7	6.20
Pima Egyptian and Acala Upland, from bulk seed.	{ 2, 4, 6 2, 4, 6	59 59	Diff. (2)-(1).....	+ .100 ± .011	+ .076 ± .012	+ .2581	+ .024 ± .013		
			Diff. $V_H$ .....	9.45	6.46		1.94		
			First (1).....	5.257 ± .009	5.302 ± .008	+ .0475	-.045 ± .011	3.96	2.86
			Second (2).....	5.405 ± .008	5.444 ± .009	+ .5271	-.039 ± .008	4.64	2.75
			Diff. (2)-(1).....	+ .148 ± .011	+ .142 ± .012	+ .4796	+ .006 ± .014		
			Diff. $V_H$ .....	13.4	11.8		0.46		

Comparing the differences between Egyptian and Upland cotton in the first and second series of determinations, we note that the differences between the differences (Table XIII, column 8) show that the second series has a numerically somewhat larger difference<sup>17</sup> in one of the three fundamental series, row 1 and 5, and a somewhat smaller difference in the other two, rows 3 and 7 and 2, 4 and 6. The difference between these differences can hardly be considered significant in comparison with their probable errors. The ratios range from 0.5 to 3.6.

It is clear, therefore, that the differentiation between the Egyptian and the Upland types with respect to the hydrogen-ion concentration of their tissue fluids can not be asserted to become greater with the advance of the season. This result for acidity, with respect to which differentiation has been shown to exist, is therefore in agreement with the ratio of specific electrical conductivity to freezing point depression, for which no differentiation has been demonstrated between the types. It is not in agreement with the results for freezing point depression,  $\Delta$ , or specific electrical conductivity,  $\kappa$ , both of which showed an increase in the differences between the Egyptian and Upland cotton with the advance of the season.

In referring to the advance of the season, we must remember that there are but two groups of data upon which conclusions concerning seasonal changes may be based. Caution must, therefore, be used in attributing a seasonal significance to these results. The term is used here merely as a means of conveniently describing the actual facts.

#### PRESENTATION AND ANALYSIS OF DATA FOR HYBRIDS

The frequency distributions of the various physicochemical measurements made upon the hybrid in 1921 have been presented in tables VI-IX. For a more detailed comparison of the properties of the fluids of hybrid and parental forms we must resort to a consideration of the differences in the individual constants and in the statistical constants derived from these individual measurements.

The results for the preliminary measurements made in 1920 will first be given. The data of the more detailed studies made in 1921 will then be presented in the form of statistical constants.

#### PRELIMINARY STUDY IN 1920

We were able to secure the following determinations:

Acala  $\times$  Pima  $F_1$  (29)<sup>18</sup> was taken on August 14. While not immediately associated with samples (1)=Egyptian and (2)=Upland, it may be most nearly compared with them. On August 18 Pima Egyptian  $\times$  Upland  $F_1$  (30) was taken between two rows of Pima (31). On the same date Pima Egyptian  $\times$  Upland  $F_1$  plants (32) were also taken in close proximity to Holdon Upland plants (33). On August 22 Pima Egyptian  $\times$  Upland  $F_1$  (34) was taken in comparison with Pima Egyptian (No. 25) and Holdon Upland (No. 26). In the same border we were so fortunate as to obtain a progeny of Acala  $\times$  Pima  $F_1$  (35) lying between

<sup>17</sup> Since the differences between the two types of cotton in column 8 are so taken as to have a negative sign when  $P_H$  is lower for Egyptian than for Upland cotton, a negative sign for the difference between the differences indicates that the difference is numerically larger in the second series of determinations.

<sup>18</sup> The numbers in parentheses are those of our samples of hybrid tissues. The number of the determination on the parent type is also given for the differences. The actual constants for these parent type numbers will be found in Table I.

the progenies of the two individual parents of the hybrid, Pima (27), and Acala (28).

The constants are set forth in table XIV.

TABLE XIV.—*Physicochemical constants for the leaf tissue fluids of the F<sub>1</sub> hybrid between Egyptian and Upland cotton grown in 1920, and differences between constants for the hybrid and those for the parent types*

Type and varieties compared.	Sample number. <sup>a</sup>	Depression of freezing point, $\Delta$	Osmotic concentration in atmospheres, P.	Specific electrical conductivity, $\kappa$ .	Ratio of conductivity to depression, $\kappa/\Delta$ .
F <sub>1</sub> hybrid, Acala Upland×Pima Egyptian.....	(29)	1.124	13.53	0.03316	0.03000
Difference between F <sub>1</sub> hybrid and Pima Egyptian.....	(29)-(1)	-0.268	-3.21	-0.0564	+0.0215
Difference between F <sub>1</sub> hybrid and Acala Upland.....	(29)-(2)	-0.094	-1.12	+0.00233	+0.0468
F <sub>1</sub> hybrid, Pima Egyptian×Upland.....	(30)	1.295	15.58	0.03015	0.02328
Pima Egyptian.....	(31)	1.365	16.42	0.03162	0.02317
Difference between F <sub>1</sub> hybrid and Pima Egyptian.....	(30)-(31)	-0.070	-0.84	-0.00147	+0.0011
F <sub>1</sub> hybrid, Pima Egyptian×Upland.....	(32)	1.496	17.99	0.03132	0.02093
Holdon Upland.....	(33)	1.468	17.65	0.03222	0.02195
Difference between F <sub>1</sub> hybrid and Holdon Upland.....	(32)-(33)	+0.028	+0.34	-0.00090	-0.00102
F <sub>1</sub> hybrid, Pima Egyptian×Upland.....	(34)	1.327	15.96	0.03141	0.03874
Difference between F <sub>1</sub> hybrid and Pima Egyptian.....	(34)-(25)	-0.020	-0.24	-0.00433	-0.00263
Difference between F <sub>1</sub> hybrid and Holdon Upland.....	(34)-(26)	+0.077	+0.92	-0.00013	-0.00249
F <sub>1</sub> hybrid, Acala Upland×Pima Egyptian.....	(35)	1.087	13.08	0.02801	0.02578
Difference between F <sub>1</sub> hybrid and Pima Egyptian.....	(35)-(27)	-0.166	-1.99	-0.00408	+0.0017
Difference between F <sub>1</sub> hybrid and Acala Upland.....	(35)-(28)	-0.026	-0.32	+0.00007	+0.00068

<sup>a</sup> Constants for parental forms not given in this table appear under these numbers in Table I.

These results show that in all four comparisons between Egyptian cotton and the hybrid between Egyptian and Upland cotton the osmotic concentration is higher in the leaf-tissue fluids of the Egyptian than in those of the hybrid plants. In all four comparisons, the specific electrical conductivity indicates that there is a higher concentration of conducting electrolytes in the tissue fluids of the Egyptian than in those of the hybrid plants. In three of the four comparisons the ratio  $\kappa/\Delta$  is higher in the hybrid than in the Egyptian cotton.

The results of the comparisons between the hybrid and the Upland cottons are more irregular. Since both hybrid and Upland cottons seem to differ from Egyptian cotton in the same direction, it is only natural to find that there is not such a conspicuous difference between hybrid and Upland as between hybrid and Egyptian.

The results of this preliminary study can not be taken as conclusive. They are, however, suggestive, and fully justified the more intensive investigation undertaken in 1921.

## INVESTIGATIONS IN 1921

### COMPARISONS ON THE BASIS OF FREEZING-POINT DEPRESSION

A comparison of the freezing-point depressions of the tissue fluids of the hybrid with its two parent forms appears in Table XV.

This table (which may serve as a type for the others of this section) is prepared as follows: The forms compared with the hybrid are given

in column 1, the number of the row in the experimental field in column 2, the number of samples upon which the statistical constants are based in column 3, and the series of collections (first or second) in column 4. The average value of the freezing-point lowering for the hybrid form appears in column 5. The freezing-point lowering for the parent forms is given in column 6. The table is divided into two sections in order to distinguish between the Egyptian (upper half of table) and Upland (lower half of table) parents.

The correlation between the parents and the hybrids of the same triplet, given in column 7 of Table XV, is essential to the determination of the probable errors of the differences between the hybrid and the parent forms and will not be discussed in this place further than to note that the differences between the correlations for the first and second series show greater irregularities than those which were found in the coefficients for the two parental forms (p. 288).

Two sets of differences are given.

The first is that between the hybrid and parent forms (columns 8-10 of Table XV). The probable errors of these absolute differences have been calculated with due regard to the influence of the heterogeneity of the field and of the variations of time and atmospheric conditions upon the probable errors, i. e., by the use of the formula

$$\sigma^2_{(h-p)} = \sigma_h^2 + \sigma_p^2 - 2r_{hp}\sigma_h\sigma_p$$

where  $h$ =hybrid and  $p$ = parent forms, and the other symbols have their usual biometric significance. The ratios of the differences to their probable errors appear in column 9.<sup>19</sup>

The second set of differences comprises those between the means for the first and second series of determinations on hybrids and parents (columns 5 and 6 of Table XV), between the correlations between parents and hybrids (column 7) in the two series, and between the differences between the hybrid and the parent forms (column 8) in the first and second series, respectively. These differences are all taken with regard to signs. The ratio of these differences to their probable errors (when probable errors have been determined) are given beneath the differences.<sup>20</sup> The probable errors of the differences between the first and second collections for the hybrid and the parent forms (columns 5 and 6) have been computed with due regard to the correlation between the determinations for the first and second series, as set forth in Table XXI. The probable errors of the differences between the differences between hybrid and parent forms (column 8) have been computed with regard to the correlation between them (as given in Table XXIII) by means of a formula similar to that given in the last paragraph above.

Considering the results of the determinations (Table XV), it is clear that the differences between the hybrid and the parent form are negative in sign throughout, thus showing that the hybrid has in all comparisons a lower osmotic concentration of tissue fluids than either of the parental forms.

<sup>19</sup> These ratios have been computed from means and probable errors carried to a larger number of significant figures than it is possible to publish in these tables (Tables XV-XVIII). They are, therefore, somewhat more exact than those which may be deduced from the constants as given here.

<sup>20</sup> These ratios, like those referred to in the note immediately above, have been calculated from constants carried to a larger number of places.



TABLE XV.—Comparison of freezing-point depression,  $\Delta$ , in the  $F_1$  hybrid with that in Pima Egyptian and Meade Upland Cotton, and in the first and second series of determinations for each type, in 1921

(1) Type and variety of parent compared with hybrid.	(2) Row.	(3) N.	(4) Series or collection.	(5) Mean for hybrid.	(6) Mean for parent.	(7) Correlation between hybrid and parent.	Difference between hybrid and parent.		
							(8) Absolute difference.	(9) Diff. $F_1$ -diff.	(10) Per-centage difference.
Pima Egyptian from self-fertilized seed. Do.....	1, 5 1, 5	39	First (1).....	1.3004 $\pm$ .0148	1.4037 $\pm$ .0179	+ .8407	-.1033 $\pm$ .0097	10.7	7.36
		39	Second (2).....	1.1373 $\pm$ .0105	1.2083 $\pm$ .0113	+ .7324	-.1610 $\pm$ .0080	17.8	12.4
	3, 7 3, 7	38	Diff. (2)-(1).....	-.1631 $\pm$ .0175	-.1054 $\pm$ .0165	-.1083	-.0577 $\pm$ .0097		
		38	Diff. $F_1$ -diff.....	9.34	6.39		5.92		
Pima Egyptian from bulk seed. Do.....	1, 3, 5, 7 1, 3, 5, 7	77	First (1).....	1.2028 $\pm$ .0094	1.3572 $\pm$ .0110	+ .6002	-.0644 $\pm$ .0092	6.98	4.75
		77	Second (2).....	1.1027 $\pm$ .0100	1.2573 $\pm$ .0093	+ .7188	-.1546 $\pm$ .0072	21.3	12.3
	1, 3, 5, 7 1, 3, 5, 7	77	Diff. (2)-(1).....	-.1001 $\pm$ .0135	-.0990 $\pm$ .0122	+ .1186	-.0902 $\pm$ .0110		
		77	Diff. $F_1$ -diff.....	14.1	8.21		8.20		
Pima Egyptian combined series. Do.....	1, 3, 5, 7 1, 3, 5, 7	77	First (1).....	1.2066 $\pm$ .0087	1.3808 $\pm$ .0107	+ .7756	-.0842 $\pm$ .0068	12.4	7.00
		77	Second (2).....	1.1202 $\pm$ .0074	1.2780 $\pm$ .0075	+ .7346	-.1578 $\pm$ .0054	20.1	12.3
	1, 3, 5, 7 1, 3, 5, 7	77	Diff. (2)-(1).....	-.1764 $\pm$ .0111	-.1028 $\pm$ .0103	-.0410	-.0736 $\pm$ .0074		
		77	Diff. $F_1$ -diff.....	15.9	9.98		10.0		
Meade Upland from self-fertilized seed. Do.....	1, 5 1, 5	39	First (1).....	1.3004 $\pm$ .0148	1.3528 $\pm$ .0137	+ .7085	-.0524 $\pm$ .0091	5.77	3.87
		39	Second (2).....	1.1373 $\pm$ .0105	1.2122 $\pm$ .0107	+ .8149	-.0749 $\pm$ .0065	11.6	6.18
	3, 7 3, 7	38	Diff. (2)-(1).....	-.1631 $\pm$ .0175	-.1406 $\pm$ .0175	+ .0164	-.0225 $\pm$ .0101		
		38	Diff. $F_1$ -diff.....	9.34	8.66		2.21		
Meade Upland from bulk seed. Do.....	1, 3, 5, 7 1, 3, 5, 7	77	First (1).....	1.2066 $\pm$ .0087	1.3515 $\pm$ .0125	+ .6175	-.0587 $\pm$ .0099	5.90	4.34
		77	Second (2).....	1.1027 $\pm$ .0100	1.2010 $\pm$ .0082	+ .6670	-.0983 $\pm$ .0076	12.9	8.18
	1, 3, 5, 7 1, 3, 5, 7	77	Diff. (2)-(1).....	-.1001 $\pm$ .0135	-.1595 $\pm$ .0140	+ .0495	-.0396 $\pm$ .0112		
		77	Diff. $F_1$ -diff.....	14.1	10.7		3.54		
Meade Upland combined series. Do.....	1, 3, 5, 7 1, 3, 5, 7	77	First (1).....	1.2066 $\pm$ .0087	1.3522 $\pm$ .0093	+ .7291	-.0556 $\pm$ .0066	8.35	4.11
		77	Second (2).....	1.1027 $\pm$ .0074	1.2067 $\pm$ .0068	+ .7477	-.0865 $\pm$ .0051	17.1	7.17
	1, 3, 5, 7 1, 3, 5, 7	77	Diff. (2)-(1).....	-.1764 $\pm$ .0111	-.1455 $\pm$ .0112	+ .0186	-.0309 $\pm$ .0075		
		77	Diff. $F_1$ -diff.....	15.9	12.9		4.12		

Since (1) the hybrid is not intermediate between the two parents with respect to the freezing point depression of its leaf tissue fluids but is characterized by a lower osmotic concentration than either of the parental forms, and since (2) the Egyptian parent is characterized by a higher osmotic concentration than the Upland parent, it is inevitable that the difference between the hybrid and the Egyptian parent should be far greater than that between the hybrid and the Upland parent. This is clearly evident in both the average value of the differences and in the ratio of these differences to their probable errors. The differences are, however, in all cases clearly significant in comparison with their probable errors. The ratios range from 5.8 to 29.1, and therefore leave no possible doubt as to the significance of the differences.

The differences between the first and second series of Egyptian and Upland cottons have been discussed above (p. 290). The comparisons of the constants for the first and second collection of hybrids (column 5 of Table XV) shows that in the heterozygous plants, as well as in the homozygous individuals of the parental strains, the second collection is characterized by a lower average osmotic concentration than the first. A remarkable fact in regard to these determinations is that in each of the six possible comparisons between the hybrid and the parental forms the hybrid shows greater decrease in osmotic concentration between the first and second collections than do either of the parent forms.<sup>21</sup>

A comparison of the differences between the hybrid and the parent forms in the first and second series,  $H_1-E_1$ ,  $H_1-U_1$ ,  $H_2-E_2$ , and  $H_2-U_2$  by means of the differences between them  $(H_2-E_2)-(H_1-E_1)$ ,  $(H_2-U_2)-(H_1-U_1)$  is possible on the basis of the values in column 8 of Table XV. These show that in every instance the difference is larger in the second series than in the first.<sup>22</sup> Thus the differentiation between the parent and the hybrid individuals tends to become greater with the advance of the season, just as the differentiation of the Egyptian and Upland forms has been shown to become larger. This rule holds, notwithstanding the fact that the actual values of the osmotic concentration are in all cases (in both hybrid and parental individuals) lower in the second series than in the first.

Comparing the seasonal increases in the numerical magnitudes of the differences between parent and hybrid forms with their probable errors, calculated by the method indicated on page 290, we note ( $\text{Diff}/E_{\text{diff}}$ , in column 8 of Table XV) that in general they may be considered statistically significant.

The percentage differences (in the final column of Table XV) have been calculated with the constant for the parental form as a base. In the comparisons between hybrid and Egyptian cotton, it appears that the hybrid has an osmotic concentration from 4.8 to 7.4 per cent lower than that of the Egyptian parent in the first series of determinations. In the second series of determinations the hybrid is characterized by an osmotic concentration of about 12 per cent lower than that of the

<sup>21</sup> A possible explanation of this difference may be found in the fact that there was but a single plant of the hybrid in each instance. It is possible that because of the necessity for a somewhat closer picking of leaves in the case of the single hybrid plant, the average age of the leaves may have been somewhat less in the second collection than in the case of the controls. This is, however, merely a suggested possibility. A solution of the problem must await further investigation.

<sup>22</sup> Since the differences between parent and hybrid forms are so taken that a negative sign indicates a lower value of freezing-point depression in the hybrid than in the parent, and since consistency in treatment throughout the paper demands the regarding of signs in taking the differences between the differences in the first and second series of determinations, a negative sign for the differences between the differences indicates that the differences between parent and hybrid are greater in the second series than in the first.

Egyptian plants growing beside it in the field. The hybrid has an osmotic concentration of its leaf-tissue fluids from 3.9 to 4.3 per cent lower than that of the Upland parent in the first series and from 6.2 to 8.2 per cent lower in the second series of determinations.

#### COMPARISON ON THE BASIS OF SPECIFIC ELECTRICAL CONDUCTIVITY

The comparison of the specific electrical conductivities<sup>23</sup> of the tissue fluids of the hybrids with their two parent forms is made in Table XVI, which is constructed on the same plan as Table XV.

The results are uniform throughout in showing a lower specific electrical conductivity in the hybrid individual than in either the Egyptian or the Upland parent.

The differences between the hybrid and either the self-fertilized or the bulk Pima cultures are relatively large, the averages for the hybrid ranging from 0.001409 to 0.002700 reciprocal ohm<sup>23</sup> lower than those for the Pima controls. These differences are from 4.3 to 17.3 times as large as their probable errors. For the whole series (rows 1, 3, 5, and 7), the specific electrical conductivity of the hybrid is lower by 0.002016 reciprocal ohm in the first series and by 0.002690 reciprocal ohm in the second series. These differences are 9.9 and 17.3 times as large as their respective probable errors.

Since the Upland cottons are characterized by leaf-tissue fluids of lower specific electrical conductivity than those of Egyptian cottons (p. 291), the difference between the hybrid and Upland parent is necessarily much smaller than that between the hybrid and the Egyptian parent. While the differences in all cases indicate a lower electrical conductivity in the tissue fluids of the hybrid than in those of its Upland parent, they can not in general be considered significant in comparison with their probable errors.

The differences between the hybrid and the Pima parent (Table XVI, column 8) are greater<sup>24</sup> in the second series of determinations than in the first series. This is in agreement with the results for osmotic concentration of hybrid and parent forms discussed above. It is also in conformity with the results of a comparison of the differentiation of the parental types.

In the comparison between the hybrid and the Upland parent, where the differences are necessarily smaller, the differences between the hybrid and the parent form are smaller in the second series of determinations.

#### COMPARISON ON THE BASIS OF THE RATIO OF SPECIFIC ELECTRICAL CONDUCTIVITY TO FREEZING-POINT DEPRESSION

The comparisons are made in Table XVII.

In the first series of determinations the differences between hybrid and parent are in part positive and in part negative in sign. They are generally small, and can not be considered statistically significant in comparison with their probable errors.

<sup>23</sup> In the tables these are given in terms of reciprocal ohms $\times 10^6$ .

<sup>24</sup> Note that since the differences between hybrid and parent form have negative signs, the law of signs requires that the differences between the differences have the negative sign when the differences are larger in the second series.

TABLE XVI.—Comparison of specific electrical conductivity,  $\kappa \times 10^6$ , in the *F*<sub>1</sub> hybrid with that in *Pima Egyptian* and *Meade Upland* cotton, and in the first and second series of determinations for each type, in 1921

(1) Type and variety of parent compared with hybrid.	(2) Row.	(3) N.	(4) Series or collection.	(5) Mean for hybrid.	(6) Mean for parent.	(7) Correlation between hybrid and parent.	Difference between hybrid and parent.		
							(8) Absolute difference.	(9) Diff. $E_{\text{diff}}$ .	(10) Per-centage difference.
<i>Pima Egyptian</i> from self-fertilized seed. Do.....	1, 5	39	First (1).....	30645 $\pm$ 235	33251 $\pm$ 300	+ .6830	-2606 $\pm$ 221	11.8	7.84
	1, 5	39	Second (2).....	27964 $\pm$ 211	30643 $\pm$ 237	+ .5774	-2679 $\pm$ 207	12.9	8.74
			Diff. (2)-(1).....	-2681 $\pm$ 311	-2608 $\pm$ 331	- .1056	-73 $\pm$ 256		
<i>Pima Egyptian</i> from bulk seed. Do.....	3, 7	38	Diff. $E_{\text{diff}}$ .....	8.61	7.87		0.29		
	3, 7	38	First (1).....	30666 $\pm$ 231	32074 $\pm$ 291	+ .2247	-1408 $\pm$ 328	4.30	4.39
	3, 7	38	Second (2).....	26946 $\pm$ 250	29646 $\pm$ 202	+ .4141	-2700 $\pm$ 232	11.6	9.11
<i>Pima Egyptian</i> , combined series. Do.....			Diff. (2)-(1).....	-3720 $\pm$ 303	-2428 $\pm$ 339	+ .1894	-1202 $\pm$ 333		
			Diff. $E_{\text{diff}}$ .....	12.2	7.15		3.88		
	1, 3, 5, 7	77	First (1).....	30955 $\pm$ 164	32071 $\pm$ 216	+ .4490	-2016 $\pm$ 205	9.85	6.17
<i>Meade Upland</i> from self-fertilized seed. Do.....	1, 5	39	Second (2).....	27461 $\pm$ 159	30151 $\pm$ 161	+ .5281	-2690 $\pm$ 155	17.3	8.92
	1, 5	39	Diff. (2)-(1).....	-3194 $\pm$ 221	-2520 $\pm$ 230	+ .0791	-674 $\pm$ 217		
			Diff. $E_{\text{diff}}$ .....	14.5	11.0		3.11		
<i>Meade Upland</i> from bulk seed. Do.....	3, 7	38	First (1).....	30645 $\pm$ 235	32041 $\pm$ 261	+ .5557	-1396 $\pm$ 235	5.95	4.35
	3, 7	38	Second (2).....	27964 $\pm$ 211	28056 $\pm$ 222	+ .3109	-92 $\pm$ 254	0.36	0.33
			Diff. (2)-(1).....	-2681 $\pm$ 311	-3085 $\pm$ 355	- .2448	+1304 $\pm$ 317		
<i>Meade Upland</i> , combined series. Do.....			Diff. $E_{\text{diff}}$ .....	8.61	11.2		4.11		
	1, 3, 5, 7	77	First (1).....	30666 $\pm$ 231	31141 $\pm$ 253	+ .3179	-475 $\pm$ 283	1.68	1.53
	1, 3, 5, 7	77	Second (2).....	26946 $\pm$ 250	27402 $\pm$ 186	+ .1599	-456 $\pm$ 269	1.69	1.66
<i>Meade Upland</i> , combined series. Do.....			Diff. (2)-(1).....	-3720 $\pm$ 303	-3739 $\pm$ 317	- .1670	+19 $\pm$ 399		
			Diff. $E_{\text{diff}}$ .....	12.2	11.8		0.05		
	1, 3, 5, 7	77	First (1).....	30955 $\pm$ 164	31597 $\pm$ 185	+ .4343	-942 $\pm$ 187	5.04	2.98
<i>Meade Upland</i> , combined series. Do.....	1, 3, 5, 7	77	Second (2).....	27461 $\pm$ 159	27733 $\pm$ 147	+ .2671	-272 $\pm$ 186	1.46	0.98
			Diff. (2)-(1).....	-3194 $\pm$ 221	-3864 $\pm$ 239	- .1672	+670 $\pm$ 250		
			Diff. $E_{\text{diff}}$ .....	14.5	16.2		2.62		

In the second series of determinations, the differences are positive in all cases, and in all cases conspicuously larger than in the first series. They are from 4.7 to 13 times as large as their probable errors. There can be no reasonable doubt of the significance of these differences.

The increase in the difference between the hybrid and the parental form (either Upland or Egyptian) from the first to the second series of determinations is from 2.8 to 6.6 times as large as its probable error. ( $\text{Diff.}/E_{\text{diff.}}$  in column 8.) The difference between the hybrid and the parental form with respect to the ratio of specific electrical conductivity to freezing point lowering may therefore be confidently asserted to increase from the first to the second series of determinations.

A comparison of the first and second series of determinations with respect to the ratio of  $\kappa$  to  $\Delta$  in the hybrids (Table XVII, column 5) and in the two cultures of the parent forms (column 6) shows that in the hybrid the ratio is higher in the second than in the first series of determinations. All of the differences are over three times as large as their probable errors, and may reasonably be regarded as statistically significant.

In the parent forms, both Egyptian and Upland, the ratio of conductivity to freezing-point depression is lower in the second than in the first series of determinations. Thus the parent forms are quite different from the hybrids in this regard. It must be noted, however, that the differences are small in the case of the parent plants, and can not be considered individually significant in comparison with their probable errors.

Expressed in relative terms (Table XVII, column 10) the differences show that in the first series of determinations the ratio is on the average from -0.01 to +2.7 per cent higher in the hybrid than in either the Egyptian or Upland parent form. In the second series the ratio is from 3.7 to 7.1 per cent higher in the heterozygous than in the homozygous plants.

The fact that the ratio in the hybrid increases from the first to the second series of determinations, whereas that in both parent forms decreases accounts for the higher percentage difference between the hybrid and each of its parent forms (column 10) in the second as compared with the first series.

#### COMPARISON ON THE BASIS OF HYDROGEN-ION CONCENTRATION

A comparison of the acidity of the leaf tissue fluids of the hybrid with that of the parent form is made in Table XVIII.

The difference column (column 8) shows at once that the hybrid is characterized by a larger value of  $P_H$  than is that of the Egyptian parental form. In the comparison between the hybrid and the Upland parent exactly the reverse is true; the hybrid has in each series a numerically smaller average value of  $P_H$  than has the Upland parent form.

The differences are in all but two cases over two and a half times as large as their probable errors. These figures and the general consistency of the results for the various series are strong evidences for the soundness of the conclusions drawn.

Thus the hybrid is intermediate in acidity between the two parental forms.

XVII.—Comparison of ratio of specific electrical conductivity to freezing-point depression,  $\kappa/\Delta \times 10^6$ , in the F<sub>1</sub> hybrid with that in *Pima Egyptian* and *Meade Upland* cotton, and in the first and second series of determinations for each type, in 1921

(1) Type and variety of parent compared with hybrid.	(2) Row.	(3) N.	(4) Series or collection.	(5) Mean for hybrid.	(6) Mean for parent.	(7) Correlation between hybrid and parent.	Difference between hybrid and parent.		
							(8) Absolute difference.	(9) Diff. $\kappa/\Delta$ .	(10) Per-centage difference.
<i>Pima Egyptian</i> from self-fertilized seed. Do.....	1, 5	39	First (1).....	23706 ± 208	23812 ± 172	+0.4624	-106 ± 109	0.53	0.45
	1, 5	39	Second (2).....	24042 ± 130	23669 ± 173	+0.1035	+973 ± 206	4.73	4.11
<i>Pima Egyptian</i> from bulk seed. Do.....	3, 7	38	Diff. (2)-(1).....	+936 ± 244	-143 ± 194	-0.3589	3.95		
	3, 7	38	Diff. $\kappa/\Delta$ .....	3.83	0.74		+102 ± 232	0.44	0.44
<i>Pima Egyptian</i> combined series. Do.....	1, 3, 5, 7	77	First (1).....	23756 ± 160	23654 ± 160	-0.0547	+870 ± 140	6.22	3.68
	1, 3, 5, 7	77	Second (2).....	24487 ± 156	23617 ± 141	+0.5578	+768 ± 271		
<i>Meade Upland</i> from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+731 ± 236	-37 ± 171	+0.6125	2.83		
	1, 5	39	Diff. $\kappa/\Delta$ .....	3.10	0.21		-3 ± 153	0.02	0.01
<i>Meade Upland</i> from bulk seed. Do.....	3, 7	38	First (1).....	23731 ± 131	23734 ± 118	+0.2575	+922 ± 125	7.37	3.90
	3, 7	38	Second (2).....	24566 ± 101	23643 ± 112	+0.3158	+925 ± 195		
<i>Meade Upland</i> combined series. Do.....	1, 3, 5, 7	77	Diff. (2)-(1).....	+835 ± 170	-91 ± 130	+0.0613	4.75		
	1, 3, 5, 7	77	Diff. $\kappa/\Delta$ .....	4.91	0.70		-47 ± 200	0.24	0.20
<i>Meade Upland</i> from self-fertilized seed. Do.....	1, 5	39	First (1).....	23706 ± 208	23753 ± 141	+0.3898	+1447 ± 212	6.82	6.24
	1, 5	39	Second (2).....	24642 ± 130	23195 ± 151	-0.1345	+1494 ± 249		
<i>Meade Upland</i> from bulk seed. Do.....	3, 7	38	Diff. (2)-(1).....	+936 ± 244	-558 ± 200	-0.5243	6.00		
	3, 7	38	Diff. $\kappa/\Delta$ .....	3.83	2.79		+617 ± 244	2.53	2.67
<i>Meade Upland</i> combined series. Do.....	1, 3, 5, 7	77	First (1).....	23756 ± 160	23139 ± 213	+0.1692	+1023 ± 209	7.76	7.10
	1, 3, 5, 7	77	Second (2).....	24187 ± 156	22864 ± 168	+0.1675	+1066 ± 343		
<i>Meade Upland</i> from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+731 ± 2	-275 ± 284	-0.0017	2.93		
	1, 5	39	Diff. $\kappa/\Delta$ .....	3.10	0.97		+281 ± 159	1.76	1.20
<i>Meade Upland</i> from bulk seed. Do.....	3, 7	38	First (1).....	23731 ± 131	23450 ± 129	+0.2522	+1334 ± 118	13.0	6.67
	3, 7	38	Second (2).....	24566 ± 101	23632 ± 114	+0.0495	+1253 ± 191		
<i>Meade Upland</i> combined series. Do.....	1, 3, 5, 7	77	Diff. (2)-(1).....	+835 ± 170	-418 ± 173	-0.2117	6.56		
	1, 3, 5, 7	77	Diff. $\kappa/\Delta$ .....	4.91	2.42				

TABLE XVIII.—Comparison of hydrogen-ion concentration,  $P_H$ , in the  $F_1$  hybrid with that in Pima Egyptian and Meade Upland cotton, and in the first and second series of determinations for each type in 1921

(1) Type and variety of parent compared with hybrid.	(2) Row.	(3) N.	(4) Series or collection.	(5) Mean for hybrid.	(6) Mean for parent.	(7) Correlation between hybrid and parent.	Difference between hybrid and parent.		
							(8) Absolute difference.	(9) Diff. $P_{diff}$ .	(10) Per-centage difference.
Pima Egyptian from self-fertilized seed Do.....	1, 5	39	First (1).....	5.316±.011	5.245±.012	+ .2735	+ .071±.014	5.22	4.40
	1, 5	39	Second (2).....	5.392±.012	5.335±.008	+ .0175	+ .057±.010	5.79	3.74
Pima Egyptian from bulk seed Do.....	3, 7	38	Diff. (2)-(1).....	+ .076±.018	+ .090±.014	+ .3440	- .014±.017		
	3, 7	38	Diff. $P_{diff}$ .....	4.29	6.26		0.87		
Pima Egyptian combined series Do.....	1, 3, 5, 7	77	First (1).....	5.298±.011	5.260±.011	+ .4594	+ .029±.011	2.59	1.82
	1, 3, 5, 7	77	Second (2).....	5.395±.013	5.378±.012	+ .7333	+ .017±.009	1.77	1.15
Meade upland from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+ .097±.017	+ .109±.015	+ .2739	- .012±.015		
	1, 5	39	Diff. $P_{diff}$ .....	5.59	7.12		0.80		
Meade upland combined series. Do.....	1, 3, 5, 7	77	First (1).....	5.307±.008	5.257±.008	+ .3440	+ .050±.009	5.60	3.12
	1, 3, 5, 7	77	Second (2).....	5.393±.009	5.357±.007	+ .0598	+ .036±.007	5.34	2.40
Meade upland from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+ .086±.012	+ .100±.011	+ .3149	- .014±.011		
	1, 5	39	Diff. $P_{diff}$ .....	6.95	9.45		1.21		
Meade upland combined series. Do.....	1, 3, 5, 7	77	First (1).....	5.316±.011	5.346±.012	+ .1243	- .030±.015	1.96	1.98
	1, 3, 5, 7	77	Second (2).....	5.392±.012	5.455±.009	+ .3207	- .063±.013	4.91	4.48
Meade upland from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+ .076±.018	+ .109±.014	+ .1964	- .033±.020		
	1, 5	39	Diff. $P_{diff}$ .....	4.29	7.90		1.74		
Meade upland combined series. Do.....	1, 3, 5, 7	77	First (1).....	5.298±.011	5.396±.012	+ .2278	- .098±.014	6.86	6.60
	1, 3, 5, 7	77	Second (2).....	5.395±.013	5.436±.012	+ .8285	- .041±.007	5.77	2.88
Meade upland from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+ .097±.017	+ .040±.018	+ .6007	+ .057±.016		
	1, 5	39	Diff. $P_{diff}$ .....	5.59	2.23		3.60		
Meade upland combined series. Do.....	1, 3, 5, 7	77	First (1).....	5.307±.008	5.370±.009	+ .1500	- .063±.011	5.90	4.23
	1, 3, 5, 7	77	Second (2).....	5.393±.009	5.446±.007	+ .5932	- .053±.007	7.57	3.75
Meade upland from self-fertilized seed. Do.....	1, 5	39	Diff. (2)-(1).....	+ .086±.012	+ .076±.012	+ .4432	+ .010±.013		
	1, 5	39	Diff. $P_{diff}$ .....	6.95	6.37		0.82		

In the determination of the percentage differences between the  $P_H$  of the hybrid and that of the parent, we have considered that the proper base for the calculation of a relative difference is the deviation of the value of  $P_H$  from that which may be taken as indicating neutrality. Thus in the calculation of these relative differences we have used the formula

$$\frac{100 (H - P)}{6.860 - P},$$

where  $H$  and  $P$  represent the average  $P_H$  values for parent,  $P$ , and hybrid,  $H$ , respectively. This is in agreement with the practice in comparing the two parental types.

The percentage differences calculated in this way range from 1.1 to 6.7.

Turning to the comparison of the first and second sets of determinations on the hybrid plants, we note that in all cases the average value of  $P_H$  is higher in the second series than it is in the first. Thus the tissue fluids in the second series of determinations are more nearly neutral than in the first series. These values range from 4.29 to 6.95 times as large as their probable errors. They may be considered unquestionably significant.

Concerning this result we have no explanation to offer. Caution must be observed in interpreting it as a regular developmental change, since in an irrigated crop grown under the severe conditions of the South-western deserts many factors must vary from one series of determinations to another. An interpretation of the differences in acidity should be one of the purposes to be considered in the planning of any future investigations in this field.

Turning to the correlations (Table XVIII, column 7), we note that the coefficients measuring the relationship between the hydrogen-ion concentration of the hybrid and of the plants of the parental form growing beside it in the fields are in all cases positive in sign. This result substantiates the findings in the comparison of the two types of cotton (Table XIII, p. 297), which shows clearly that acidity is to some extent determined by the varying conditions of the soil or of the atmosphere at the time at which the samples were taken.

The second series of correlations is numerically larger than the first. This indicates either (a) that the plants have become more closely related to their substratum or (b) that their atmospheric environment differs more widely from time to time in the second series than in the first, or (c) that the determinations in the second series were made with a considerably higher order of precision than those of the first series.

These results indicate clearly the importance of further investigations along these lines.

Comparing the differences between the hybrid and the parent form in the first series with that in the second series (Table XVIII, column 8) we find that in the comparison with Egyptian cotton the differences are in all cases greater in the first than in the second series, but these differences can not be considered significant in relation to their probable errors. In the comparison with Upland cotton the differences are numerically greater in the first series for rows 3 and 7 and for rows 1, 3, 5, and 7, but numerically greater in the second series for rows 1 and 5.



## DISCUSSION OF RESULTS

The data presented in the foregoing sections lead to definite conclusions as far as matters of fact concerning the physicochemical properties investigated are concerned. The consistency of results for the two years, the close concordance of the results for the various subseries in the comprehensive experiment of 1921, and the large size of the differences when considered in comparison with their probable errors, leave no possible doubt as to the existence of differences between the tissue fluids of the Egyptian and Upland cotton, and between the  $F_1$  hybrid and the two parental types, with respect to the physicochemical properties investigated.

This represents a distinct advance in our working knowledge of the characteristics of a highly important economic plant. The biologist would, however, like to see some explanation of the demonstrated differences in terms of more fundamental biological phenomena. He would at least like to be sure that every possible biological source of error is eliminated.

We may, therefore, review again certain possible explanations of the differences which have been demonstrated.

## REVIEW OF BIOLOGICAL SOURCES OF ERROR

The probable errors of random sampling have been so reduced by the number of data accumulated that the conclusions are not open to criticism on the grounds of the results being due to chance.

Differences between the plant forms due to differences in distribution over a field characterized by diversity in soil conditions have been fully eliminated, unless we assume that the forms are remarkably different in the depth of root penetration, and that the soil is sufficiently different at the various levels to influence the physicochemical properties in a different manner.

At present we have no adequate information on this point. It is a question to which we hope to give some attention later.

A possible source of error—and one which seems largely unavoidable during the stages of development preceding maturity—is to be found in the differences in the growth habit of the two groups (Egyptian and Upland) of cotton. This renders the collecting of wholly comparable leaves somewhat difficult, but the greatest care was exercised in this work. Ultimately it may be possible to refer the differences here demonstrated to some more fundamental physiological or morphological phenomenon involving stage of development, but at present we are unable to do so.

## DIFFERENCES BETWEEN THE FIRST AND SECOND SERIES OF DETERMINATIONS

We have now to consider certain outstanding features of these series of constants.

Conspicuous among these is the fact that all the constants differ between the first and the second series. This fact may be established by the comparison of the actual differences in the eight fundamental tables of constants (Tables X to XIII and XV to XVIII) with their probable errors. For convenience of reference and to facilitate the comparison of the various

constants, these differences have been expressed as percentages of the average value of the first determination in Table XIX. In this table the percentages are calculated by using the actual value of the first constant as a base, except in the case of  $P_H$ , where the deviation of the first constant from neutrality, taken as 6.86, has been used.

A comparison between the first and second series is facilitated by determining averages for all the differences for the same type of plants (Egyptian, Upland, or hybrid). In determining such averages the percentage differences for the individual subcultures may be used, but some correction must be made (by weighting) for the differences in the size of the subcultures. The averages of the change from first to second series in Table XIX have been computed for the Egyptian and Upland plants by weighting the constants for rows 1 and 5 and for rows 3 and 7 by two and that for rows 2, 4, and 6 by three and taking 7 as  $N$  in the determinations of the average. The averages for the hybrids have been calculated without weighting.

TABLE XIX.—Percentage difference in average value of constants for first and second series of determinations in Pima Egyptian, Meade and Acala Upland, and  $F_1$  hybrid cotton, in 1921

Type and variety of cotton and nature of seed planted.	Rows.	Freezing-point depression, $\Delta$	Specific electrical conductivity, $\kappa$	Ratio of conductivity to depression, $\kappa/\Delta$	Hydrogen-ion concentration, $P_H$
Pima Egyptian from self-fertilized seed	1, 5	-7.51	-7.84	-0.60	+5.57
Pima Egyptian from bulk seed	3, 7	-7.36	-7.57	-1.16	+6.85
Pima Egyptian combined series	1, 3, 5, 7	-7.44	-7.71	-1.38	+6.24
Pima Egyptian from bulk seed	2, 4, 6	-4.45	-6.39	-2.15	+9.23
Average		-6.16	-7.14	-1.14	+7.50
Meade Upland from self-fertilized seed	1, 5	-10.39	-12.44	-2.35	+7.20
Meade Upland from bulk seed	3, 7	-11.14	-12.01	-1.19	+2.73
Meade Upland combined series	1, 3, 5, 7	-10.76	-12.23	-1.78	+5.10
Acala Upland from bulk seed	2, 4, 6	-9.05	-11.28	-2.68	+9.05
Average		-10.03	-11.82	-2.16	+6.72
$F_1$ Hybrid between Egyptian and Upland	1, 5	-12.54	-8.75	+3.95	+4.92
Do	3, 7	-14.70	-12.13	+3.08	+6.21
Do	1, 3, 5, 7	-13.60	-10.42	+3.52	+5.54
Average		-13.63	-10.44	+3.51	+5.57

Both osmotic concentration as measured by the depression of the freezing point,  $\Delta$ , and specific electrical conductivity in reciprocal ohms,  $\kappa$ , are lower in the second series than the first. The values of  $P_H$  are higher in the second than in the first series, but since higher values of  $P_H$  indicate lower concentrations of the hydrogen ion, it is clear that the concentration of the hydrogen ion is also lower in the second series than in the first. Thus the concentration of total solutes (molecules and ions), of all conducting solutes, and of hydrogen ions, is less in the second series than in the first.

The ratio of specific electrical conductivity to freezing-point depression is lower in the second series of determinations than in the first series for both Egyptian and Upland cottons, but the percentage differences are small. Just the reverse is found in the hybrid. The explanation of these differences between the hybrids and the parent forms must await the results of further investigations.

Two possible explanations of the decrease in osmotic concentration, specific electrical conductivity, and hydrogen-ion concentration from the first to the second series will at once suggest themselves.

Soil moisture may have been greater or, due to the retardation of growth toward the end of the season, the requirements for soil moisture may have been less, in the second than in the first series. As a consequence of either of these conditions, the turgidity of the leaves may have been somewhat higher in the second series. Thus through the dilution effect of higher turgidity the measures of the concentration of solutes might be lower in the second series.

It is quite conceivable that the rapidly developing bolls made much higher demands upon the solutes of the leaf tissue fluids in the second period than in the first and that the lower values of both the osmotic concentration and specific electrical conductivity in the second period may have been due primarily to the withdrawal of solutes from the leaf by the developing fruits.

It is obviously impossible to determine with certainty which of these suggested hypotheses is the more logical, or what other explanation may be the true one, without special investigation. It is worth while to note, however, that the decrease in hydrogen-ion concentration can hardly be explained by the simple assumption of relatively higher turgidity in the second series of determinations. The organic acids are in general so weakly ionized that dilution has but little influence on the concentration of the hydrogen-ion. Since dilution is a highly improbable explanation of the change in the hydrogen-ion concentration, it can hardly be assumed to be the most probable explanation of changes in the value of  $\Delta$  and  $\kappa$ .

Some light may perhaps be thrown upon the problem by considering in a comparative way the percentage difference between the first and second series.

A comparison of the percentage changes in Egyptian and Upland cotton as given in Table XIX shows that the decrease in the concentration of total solutes and of electrolytes from the first to the second series of determinations is greater in the Upland than in the Egyptian cotton. The average value of the change in freezing-point depression,  $\Delta$ , is 6.16 per cent in Egyptian and 10.03 per cent in Upland cotton. The average change in specific electrical conductivity,  $\kappa$ , is 7.14 in Egyptian and 11.82 in Upland cotton. This result has an important bearing upon the suggested hypothesis that the decrease in osmotic concentration and electrical conductivity from the first to the second series of determinations is due to the rapid withdrawal of solutes from the leaves by the developing bolls. If the factor of the withdrawal of solutes by the developing bolls is important enough to account for the decrease in the concentrations of total solutes and electrolytes from the first to the second series, it might reasonably be expected to account for certain of the differences between the various kinds of plants. We have no quantitative measure of the relative number of developing bolls in the various series of plants in the first and second series, but the general impression derived from an inspection of the two types of cotton in the field is that at the time of the first series of determinations the Upland plants had relatively more well developed bolls than the Egyptian plants, while by the time the second series of determination was made, the slower-developing Egyptian cotton had produced a more nearly comparable crop of fruits.

Now the actual results for percentage change in concentration from the first to the second series are exactly the opposite of what might be expected from the hypothesis and from the apparent facts with regard to the fruiting of the cottons. Since the Upland cotton was well into the fruiting condition when the first series of determinations was made, while the Egyptian cotton was just beginning to form bolls, one might on the assumptions made expect to find the greatest change in the sap properties of the Egyptian plants since it is in these plants that the greatest change in physiological state with respect to fruiting seems to have taken place. The results are just the reverse.

It is interesting in this connection to compare the results for the hybrid and the parent forms. The hybrid series show a greater percentage decrease in the value of  $\Delta$  than either the Upland or the Egyptian plants, and there was presumably less change in the condition with respect to fruiting between the first and the second series than in the case of the Egyptian cotton.

#### THE RELATIVE MAGNITUDES OF THE DIFFERENCE BETWEEN THE TWO SPECIES IN THE FIRST AND SECOND SERIES OF DETERMINATIONS

In the discussion of the individual constants we have noted that the difference between the Egyptian and Upland type is generally larger in the second than in the first series. This is true for freezing-point depression, specific electrical conductivity, and generally for the ratio of specific electrical conductivity to freezing-point depression. The differences between the differences in the varieties in the first and second series may be considered significant in the case of the two direct measurements, but are not sufficiently large to be regarded as certainly trustworthy in the various subseries in the case of the ratio. The results for hydrogen-ion concentration are uncertain since the signs of the differences between the differences are not wholly consistent, and only one of the differences (that for rows 3 and 7) may be considered statistically significant in comparison with its probable error.

It is a significant fact that while the second series uniformly shows a larger differentiation of the Egyptian and the Upland types for both freezing-point depression and specific electrical conductivity, the actual magnitudes of the physical constants are lower for both freezing-point depression,  $\Delta$ , and specific electrical conductivity,  $\kappa$ , in the second than in the first series. The ratio  $\kappa/\Delta$  is on the average consistently lower in the second than in the first series, but the differences between the means are small and can not be considered significant individually in comparison with their probable errors. This is also true for the hydrogen-ion concentration.

For convenience of comparison, all of the percentage differences between Egyptian and Upland cotton given in column 10 of tables X to XIII have been laid side by side in Table XX.

TABLE XX.—Comparison of percentage difference between Pima Egyptian, Meade and Acala Upland cotton, in the first and second series of determinations and for various constants, in 1921

Comparison.	Rows.	Series or collection.	Freezing-point depression, $\Delta$ .	Specific electrical conductivity, $\kappa$ .	Ratio of conductivity to depression, $\kappa/\Delta$ .	Hydrogen-ion concentration $P_H$ .
Pima Egyptian from self-fertilized seed and Meade Upland from self-fertilized seed.	1, 5 1, 5	First (1)...	+3.76	+0.77	+0.25	-6.67
		Second (2)...	+7.10	+9.22	+2.04	-8.54
		Diff. (2)-(1).	+3.34	+8.45	+1.79	-1.87
Pima Egyptian from bulk seed and Meade Upland from bulk seed.....	3, 7 3, 7	First (1)...	+0.42	+2.99	+2.23	-8.67
		Second (2)...	+4.69	+8.19	+3.29	-4.07
		Diff. (2)-(1).	+4.27	+5.20	+1.06	+4.60
Pima Egyptian and Meade Upland, combined series.....	1, 3, 5, 7 1, 3, 5, 7	First (1)...	+2.12	+3.40	+1.21	-7.53
		Second (2)...	+5.91	+8.72	+2.65	-6.29
		Diff. (2)-(1).	+3.79	+5.32	+1.44	+1.29
Pima Egyptian and Acala Upland from bulk seed.....	2, 4, 6 2, 4, 6	First (1)...	+3.10	+2.93	-0.37	-2.89
		Second (2)...	+8.31	+8.60	+0.22	-2.75
		Diff. (2)-(1).	+5.22	+5.67	+0.59	+0.14

The reasons for the larger differences in the values of freezing-point lowering and specific electrical conductivity in the second as compared with the first series are difficult to determine. Among the possibilities may be mentioned:

(1) That the greater exactness of the constants in the second series, resulting from all the collections of samples having been made by one observer, has resulted in more definite differences in the two series.

(2) That the differentiation here under consideration is dependent upon the physiological age of the organism.

These are questions which await further investigation.

#### THE CORRELATION BETWEEN THE FIRST AND THE SECOND SERIES OF DETERMINATIONS

In the preceding discussion we compared the constants for the first and second series of measurements. To determine the probable errors of the differences between the two series, it was necessary to compute the coefficients of correlation between them by considering the homologous samples of the first and second series as the two variables of a pair and the number of homologous pairs as  $N$ .

The coefficients measuring the correlation between the determinations of the first and second series appear in Table XXI. Here the varieties and the rows are arranged as in preceding tables. The headings for  $\Delta$ ,  $\kappa$ ,  $\kappa/\Delta$ , and  $P_H$  indicate the correlations between the first and second series for the values of these constants. The table is broken into three sections, the upper for the Pima Egyptian, the second for the Upland, and the lower section for the hybrid.

The correlations between the first and second series of constants must be considered from two sides—first, that of the relative magnitude of the coefficients in the two species and their hybrid; second, that of the relative magnitude of the correlations between the first and the second series of collections for the four physicochemical constants under consideration.

TABLE XXI.—Comparison of correlation between the first and second series of determinations for Egyptian and Upland cotton and their  $F_1$  hybrid, in 1921

Type, variety, and nature of seed planted.	Rows.	Correlation for depression of freezing point, $\Delta$ .		Correlation for specific electrical conductivity, $\kappa$ .		Correlation for ratio of conductivity to depression, $s/\Delta$ .		Correlation for hydrogen-ion concentration, $F_{H^+}$ .	
		$r \pm Er$	$r/Er$	$r \pm Er$	$r/Er$	$r \pm Er$	$r/Er$	$r \pm Er$	$r/Er$
Egyptian cotton:									
Pima, from selfed seed.	1, 5	$+ .4350 \pm .0876$	4.97	$+ .2553 \pm .1009$	2.53	$+ .3681 \pm .0934$	3.94	$- .0105 \pm .1080$	0.10
Pima, from bulk seed.	3, 7	$+ .2863 \pm .1005$	2.85	$+ .2401 \pm .1027$	2.40	$+ .3586 \pm .0953$	3.76	$+ .0972 \pm .1084$	0.90
Pima, combined series.	1, 3, 5, 7	$+ .4049 \pm .0643$	6.30	$+ .2854 \pm .0706$	4.04	$+ .3637 \pm .0607$	5.45	$+ .0726 \pm .0765$	0.95
Pima, from bulk seed.	2, 4, 6	$+ .4939 \pm .0664$	7.44	$+ .2703 \pm .0813$	3.32	$+ .2143 \pm .0837$	2.56	$+ .1419 \pm .0860$	1.65
Average.		$+ .4178$	.....	$+ .2591$	.....	$+ .2995$	.....	$+ .0856$	.....
Upland cotton:									
Meade, from selfed seed.	1, 5	$- .0070 \pm .1080$	0.06	$- .0785 \pm .1073$	0.73	$- .0665 \pm .1075$	0.62	$+ .1358 \pm .1060$	1.28
Meade, from bulk seed.	3, 7	$+ .1247 \pm .1077$	1.16	$- .0219 \pm .1093$	0.20	$- .0992 \pm .1083$	0.92	$- .1869 \pm .1056$	1.77
Meade, combined series.	1, 3, 5, 7	$+ .0456 \pm .0767$	0.59	$- .0200 \pm .0768$	0.26	$- .0136 \pm .0769$	0.18	$- .0627 \pm .0766$	0.82
Acacia, from bulk seed.	2, 4, 6	$- .0233 \pm .0878$	0.27	$- .1364 \pm .0861$	1.58	$- .1299 \pm .0863$	1.51	$- .0179 \pm .0879$	0.20
Average.		$+ .0236$	.....	$- .0871$	.....	$- .1030$	.....	$- .0223$	.....
Hybrid cotton:									
$F_1$ , Pima $\times$ Meade.	1, 5	$+ .0745 \pm .1074$	0.69	$+ .0276 \pm .1079$	0.26	$+ .0058 \pm .1080$	0.05	$- .1371 \pm .1060$	1.29
$F_1$ , Pima $\times$ Meade.	3, 7	$+ .0306 \pm .1093$	0.28	$+ .1114 \pm .1080$	1.03	$- .1160 \pm .1079$	1.08	$- .0647 \pm .1090$	0.59
$F_1$ , Pima $\times$ Meade.	1, 3, 5, 7	$+ .0629 \pm .0766$	0.82	$+ .0672 \pm .0765$	0.88	$- .0509 \pm .0767$	0.66	$- .1019 \pm .0761$	1.34
Average.		$+ .0525$	.....	$+ .0695$	.....	$- .0556$	.....	$- .1009$	.....

A casual glance at Table XXI will show that the Egyptian series on the one hand and the Upland and the hybrid series on the other differ materially with respect to the correlations between the first and second series of determinations. All of the coefficients for  $\Delta$ ,  $\kappa$ , and  $\kappa/\Delta$  in the Pima series are positive and in general may be considered significant in comparison with their probable errors. The comparable coefficients for the Upland series and for the hybrid plants are characterized by low values of the correlation coefficients. These may be either positive or negative in sign. They are of the same general order of magnitude as their probable errors. No one of the 21 coefficients can be considered certainly significant in comparison with its probable error.

The coefficients for hydrogen-ion concentration are low throughout, and can not be considered statistically significant in comparison with their probable errors.

Taking the averages<sup>25</sup> of the constants for the parental groups and for the hybrid groups, we note that all of the coefficients for the Upland plants are numerically less than 0.11. One of them is positive while three are negative in sign. The averages for  $\Delta$ ,  $\kappa$ , and  $\kappa/\Delta$  for the hybrid plants are less than 0.07. The average value of the two coefficients for hydrogen-ion concentration in the hybrid plants is -0.10.

Contrasted with these, we find in the Pima Egyptian series the following averages:  $r = +0.418$  for  $\Delta$ ,  $r = +0.259$  for  $\kappa$ ,  $r = +0.299$  for  $\kappa/\Delta$ , and  $r = +0.086$  for  $P_{H^+}$ . Clearly these correlations for the Egyptian series are far higher than those for the Upland series.

We have considered various possible explanations of this difference in correlation, but since actual data are wanting for the adequate substantiation or refutation of any of the suggested possibilities, it seems wisest to omit all discussions of suggested explanations until further experimental evidence, now being collected, can be analyzed.

That the behavior of the two types of plants is quite different is further suggested by a consideration of the cross correlations between the constants of the plants of the same duplet or triplet in the first and second series, as set forth in Table XXII. The coefficients in this table were determined primarily as a means of obtaining the probable errors of the differences between the first and second series of differences between the Egyptian and the Upland types. The few words of discussion are purely incidental. The problem will, we hope, receive more detailed treatment on the basis of further data now being collected.

The coefficients presented show the relationship between the first determination on the Egyptian plants of a duplet or triplet and the second determination on the Upland or hybrid plants of the same duplet or triplet; or, conversely, the constants show the correlation between the first determination on the Upland or hybrid plants of a duplet or triplet and the second determination on the Pima plants; or, finally, the coefficients show the relationship between the first determination on the Upland plants of a triplet and the second determination on the hybrid plants, or the first determination on the hybrid plants and the second determination on the Upland plants.

The first two columns of Table XXII show the plants which furnished the materials for the first and second determinations, respectively. For convenience of comparison these are arranged in pairs. The first

<sup>25</sup> The averages of the correlations have been computed for the Egyptian and the Upland plants by weighting the constants for rows 1 and 5 and for 3 and 7 by two and that for rows 2, 4 and 6 by three, and taking 7 as  $N$  in the determination of the average for the whole experiment.

TABLE XXII.—Comparison of correlation between (a) the physicochemical constants for the first series of determinations based on Upland Cotton (Meade and Acala varieties) and on the F<sub>1</sub> hybrid and those of the second series of determinations based on Egyptian cotton (Pima variety) of the same duplet or triplet; and (b) the constants for the first series of determinations based on Egyptian cotton and those of the second series of determinations based on Upland cotton or on the F<sub>1</sub> hybrid of the same duplet or triplet, in 1921

First series of determinations.	Second series of determinations.	Rows.	Correlations for depression of freezing point, Δ.		Correlations for specific electrical conductivity, κ.		Correlation for ratio of conductivity to depression, κ/Δ.		Correlation for hydrogen-ion concentration, P <sub>H</sub> .	
			r ± E <sub>r</sub>	r/E <sub>r</sub>	r ± E <sub>r</sub>	r/E <sub>r</sub>	r ± E <sub>r</sub>	r/E <sub>r</sub>	r ± E <sub>r</sub>	r/E <sub>r</sub>
Meade Upland <sup>a</sup> . Pima Egyptian.	Pima Egyptian. Meade Upland.	1, 5 1, 5	+ .3796 ± .0971 + .1321 ± .1061	3.27 1.24	— .1528 ± .1055 + .1013 ± .1040	1.44 1.83	+ .2707 ± .1001 + .0431 ± .1078	2.70 0.41	+ .0627 ± .1076 + .0283 ± .1080	0.58 0.26
		Difference.	+ .1854 ± .1438	1.28	— .3447 ± .1481	2.32	+ .2256 ± .1471	1.53	+ .0344 ± .1525	0.22
Meade Upland <sup>b</sup> . Pima Egyptian.	Pima Egyptian. Meade Upland.	3, 7 3, 7	+ .2650 ± .1017 + .1405 ± .1073	2.66 1.30	+ .2334 ± .1035 + .2185 ± .1042	2.25 2.09	+ .0972 ± .1083 + .1470 ± .1071	0.80 1.37	— .0264 ± .1033 + .0655 ± .1090	0.24 0.60
		Difference.	+ .1245 ± .1478	0.84	+ .0746 ± .1468	0.10	— .0498 ± .1523	0.33	— .0919 ± .1544	0.59
Acala Upland <sup>a</sup> . Pima Egyptian.	Pima Egyptian. Acala Upland.	2, 4, 6 2, 4, 6	+ .3461 ± .0773 — .0558 ± .0875	4.47 0.63	+ .2038 ± .0842 — .0555 ± .0875	2.42 0.63	+ .0215 ± .0878 + .0446 ± .0876	0.24 0.50	+ .0745 ± .0873 + .0434 ± .0876	0.85 0.49
		Difference.	+ .4019 ± .1168	3.44	+ .2593 ± .1214	2.13	— .0231 ± .1240	0.18	+ .0311 ± .1237	0.25
F <sub>1</sub> hybrid <sup>a</sup> . Pima Egyptian.	Pima Egyptian. F <sub>1</sub> hybrid.	1, 5 1, 5	+ .4949 ± .0903 + .0001 ± .1080	4.48 0.00	+ .1538 ± .1054 — .0366 ± .1075	1.45 0.34	+ .2937 ± .0986 + .1405 ± .1059	2.97 1.32	— .0329 ± .1079 + .1440 ± .1058	0.30 1.36
		Difference.	+ .4948 ± .1408	2.87	+ .1904 ± .1508	1.26	+ .4342 ± .1446	3.00	+ .1111 ± .1511	0.73
F <sub>1</sub> hybrid <sup>b</sup> . Pima Egyptian.	Pima Egyptian. F <sub>1</sub> hybrid.	3, 7 3, 7	+ .2344 ± .1034 + .0599 ± .1093	2.26 0.27	+ .0852 ± .1086 — .1326 ± .1075	0.78 1.23	+ .0197 ± .1093 + .2843 ± .1006	0.18 2.82	— .0732 ± .1088 + .1972 ± .1052	0.67 1.87
		Difference.	+ .2045 ± .1595	1.35	+ .2178 ± .1528	1.43	— .2646 ± .1485	1.78	— .2704 ± .1513	1.78
Meade Upland <sup>a</sup> . F <sub>1</sub> hybrid.	F <sub>1</sub> hybrid. Meade Upland.	1, 5 1, 5	— .1093 ± .1067 + .0983 ± .1070	1.02 0.91	— .3770 ± .0927 + .1532 ± .1055	4.06 1.45	— .0854 ± .1073 + .2648 ± .1004	0.76 2.63	+ .0354 ± .1079 + .1867 ± .1042	0.33 1.79
		Difference.	— .2070 ± .1511	1.37	— .5302 ± .1404	3.77	+ .1833 ± .1469	1.24	+ .2231 ± .1500	1.48
Meade Upland <sup>b</sup> . F <sub>1</sub> hybrid.	F <sub>1</sub> hybrid. Meade Upland.	3, 7 3, 7	+ .0921 ± .1085 + .1454 ± .1071	0.84 1.35	— .0215 ± .1094 + .1497 ± .1070	0.19 1.39	— .0244 ± .1079 + .0587 ± .1076	0.20 0.54	— .1590 ± .1067 + .1282 ± .1076	1.49 1.19
		Difference.	— .0533 ± .1595	0.34	— .1712 ± .1530	1.12	— .0811 ± .1523	0.53	— .0308 ± .1515	0.20

<sup>a</sup> Pima Egyptian and Meade Upland, both from self-fertilized seed.

<sup>b</sup> Pima Egyptian and Meade Upland, both from bulk seed.

<sup>c</sup> Pima Egyptian and Acala Upland, both from bulk seed.



two horizontal rows of constants, for example, show the correlations between the first collection of Meade and the second collection of Pima and the correlation between the first collection of Pima and the second collection of Meade for the same triplet.

To bring out clearly the relationships shown by these correlations it is necessary to determine the differences between the two coefficients of a pair. In determining and interpreting these differences we must bear in mind the fact that the mathematical coefficient of correlation may be either positive or negative in sign. Positive coefficients indicate that in the long run the two variables tend to deviate from the averages of their respective means in the same direction. By higher coefficient, as used in the comparison of the two coefficients of a pair in the present connection, we understand that which is numerically larger when both have the same sign, or the one which most nearly approached  $+1$  when the sign of one or both of the coefficients is negative.

The differences in Table XXII are so taken that a positive sign indicates that the correlation is higher when (a) the Egyptian determination is made in the second series and the Upland determinations made in the first series, and (b) when the Egyptian determination is made in the second series and the hybrid determination in the first series, and (c) when the hybrid determination is in the second series and the Upland determination in the first series.

Limiting our attention for the moment to the cross correlations between the two parent types (i. e., omitting the correlations in which one constant is based upon a hybrid individual), we note that the coefficients are preponderantly positive in sign, thus showing that when a first determination on one variety is compared with a second determination made at a later date on a sample of tissue of another variety drawn from the same part of the plot there will be a measurable similarity between them. Individually the constants are low and can not in general be considered significant in comparison with their probable errors.

The correlations in which one of the two variables is a constant based on a hybrid plant are very irregular and can not in general be considered significant in comparison with their probable errors.

A comparison between the correlations for the two parent types shows that in 8 of the 12 comparisons the correlations are somewhat higher when the Upland culture furnished the first sample and the Egyptian culture furnished the second sample than when the reverse is true.

We have at present no explanation to offer for this difference in the behavior of the two types of plants. The results are set forth merely as matters of fact ascertained in the determination of correlations to be used primarily in the determination of probable errors. (See p. 289.) The explanation of the facts must await further research.

The correlations between the differences between the biological forms compared in the first series of determinations and the differences between the same forms as compared in the second series of determinations are given in Table XXIII. These are presented because they have been used in the determination of the probable errors of the differences between the differences.

The second problem, that of the relative magnitude of the coefficients for the four physicochemical measurements, will be considered when the problem of the magnitudes of the correlations for the same duplet or triplet is discussed. (See p. 319.)

TABLE XXIII.—Comparison of correlation between the differences between the various types of plants (Pima Egyptian, Meade and Acala Upland and  $F_1$  hybrid cotton) in the first and second series of determinations, in 1921

Types and varieties between which differences have been taken in the first and second series of determinations.	Rows.	Correlation for depression of freezing point, $\Delta$ .		Correlation for specific electrical conductivity, $\kappa$ .		Correlation for ratio of conductivity to depression, $\kappa/\Delta$ .		Correlation for hydrogen-ion concentration, $P_n$ .	
		$r \pm E_r$	$r/E_r$	$r \pm E_r$	$r/E_r$	$r \pm E_r$	$r/E_r$	$r \pm E_r$	$r/E_r$
Pima Egyptian, from self-fertilized seed, and Meade Upland, from self-fertilized seed.....	1, 5	+ .1773 $\pm$ . 1046	1. 69	+ .1745 $\pm$ . 1047	1. 67	+ .1258 $\pm$ . 1063	1. 18	+ .0405 $\pm$ . 1078	0. 37
Pima Egyptian, from bulk seed, and Meade Upland, from bulk seed.....	3, 7	- .0201 $\pm$ . 1094	0. 18	- .1763 $\pm$ . 1060	1. 66	- .0779 $\pm$ . 1088	0. 72	- .1125 $\pm$ . 1080	1. 04
Pima Egyptian, from bulk seed, and Acala Upland, from bulk seed.....	2, 4, 6	+ .2846 $\pm$ . 0807	3. 52	+ .0449 $\pm$ . 0876	0. 51	+ .2203 $\pm$ . 0835	2. 64	+ .0073 $\pm$ . 0878	0. 08
Pima Egyptian, combined series, and Meade Upland, combined series.....	1, 3, 5, 7	+ .1306 $\pm$ . 0756	1. 72	+ .0003 $\pm$ . 0769	0. 12	+ .0450 $\pm$ . 0767	0. 50	- .0801 $\pm$ . 0764	1. 04
Pima Egyptian, from self-fertilized seed, and $F_1$ hybrid.....	1, 5	+ .4117 $\pm$ . 0897	4. 58	+ .2807 $\pm$ . 0989	2. 93	+ .0905 $\pm$ . 1071	0. 85	+ .0326 $\pm$ . 1070	0. 30
Pima Egyptian, from bulk seed, and $F_1$ hybrid.....	3, 7	+ .1231 $\pm$ . 1078	1. 14	+ .3207 $\pm$ . 0975	3. 38	- .0073 $\pm$ . 1094	0. 07	- .1322 $\pm$ . 1075	1. 22
Pima Egyptian, combined series, and $F_1$ hybrid, combined series.....	1, 3, 5, 7	+ .2881 $\pm$ . 0705	4. 08	+ .2983 $\pm$ . 0700	4. 26	+ .0182 $\pm$ . 0766	0. 24	+ .0063 $\pm$ . 0760	0. 08
Meade Upland, from self-fertilized seed, and $F_1$ hybrid.....	1, 5	+ .1816 $\pm$ . 1044	1. 73	+ .1615 $\pm$ . 1052	1. 54	+ .2679 $\pm$ . 1002	2. 67	+ .0469 $\pm$ . 1078	0. 43
Meade Upland, from bulk seed, and $F_1$ hybrid.....	3, 7	+ .2095 $\pm$ . 1046	2. 00	- .0410 $\pm$ . 1092	0. 38	- .1429 $\pm$ . 1072	1. 33	+ .0543 $\pm$ . 1091	0. 49
Meade Upland, combined series, and $F_1$ hybrid, combined series.....	1, 3, 5, 7	+ .2026 $\pm$ . 0737	2. 74	+ .0549 $\pm$ . 0766	0. 72	+ .0704 $\pm$ . 0765	0. 92	+ .0189 $\pm$ . 0768	0. 24

## THE CORRELATION BETWEEN THE MEMBERS OF THE SAME DUPLET OR TRIPLET

A casual examination of the fundamental Tables X to XIII and XV to XVIII will have shown that there is a material correlation between the sap properties of the plants of the same duplet or triplet. This correlation may, in part, be assumed to be due to substratum heterogeneity and in part to the differentiation of the individual duplets or triplets from the population as a whole by meteorological and time factors.

Further evidences of the existence of such differentiating factors have been furnished by the correlations between the first and second determinations on plants of the same duplet or triplet as set forth in Tables XXI and XXII.

It might be assumed that the coefficients of correlation between the physicochemical constants of the plants of the same duplet or triplet will furnish some measure of the susceptibility of these physicochemical constants ( $\Delta$ ,  $\kappa$ ,  $\kappa/\Delta$ , and  $P_H$ ) to the influence of (a) the relatively permanent differentiation of the substratum and (b) the transient differentiation, due to variations in water content and atmospheric conditions. The closest correlations would be expected for the characters which are more susceptible to external influences. This assumption will be valid if all of the physicochemical properties have been measured with an approximately equal degree of accuracy, so that the errors of measurement will not materially influence the correlation coefficients.

We have, therefore, prepared a comparison of the correlations between the determinations of the same duplet or triplet in the various cultures. This comparison is made in Table XXIV. The constants given are the differences between the correlation coefficients as indicated in the headings of the columns. Thus  $\Delta-\kappa$  indicates the difference between the correlation for freezing-point lowering and the correlation for specific electrical conductivity between the members of the duplets or triplets of the rows indicated. The original correlation coefficients are given in the eight fundamental tables X to XIII and XV to XVIII of constants.

The probable errors of the differences in the correlations between the various constants have not been determined, since we are not sure in how far the ordinary statistical theory of the probable error of the difference between two constants is applicable to the present case.

We have first to consider the correlation between the osmotic concentration of the plants of the same duplet or triplet in comparison with the magnitudes of the comparable coefficients for the other physicochemical properties.

The correlation coefficient for osmotic concentration,  $\Delta$ , is in every instance higher than that for specific electrical conductivity,  $\kappa$ , and in every instance higher than that for the ratio of specific electrical conductivity to freezing-point lowering,  $\kappa/\Delta$ . It is in nearly every case higher than that for hydrogen-ion concentration expressed in terms of  $P_H$ .

TABLE XXIV.—Comparison of the correlations for the same duplet or triplet in 1921, for the various physico-chemical properties, the values being the differences between the coefficients of correlation for each pair of characters

Member of duplet or triplet between which the correlation is determined.	Rows.	Difference between correlation for depression of freezing point, $\Delta$ , and specific electrical conductivity, $\kappa$ , $\Delta-\kappa$	Difference between correlation for depression of freezing point, $\Delta$ , and ratio of conductivity to depression, $\kappa/\Delta$ , $\Delta-\kappa/\Delta$	Difference between correlation for depression of freezing point, $\Delta$ , and hydrogen-ion concentration, $P_H$ , $\Delta-P_H$	Difference between correlation for specific electrical conductivity, $\kappa$ , and ratio of conductivity to depression, $\kappa/\Delta$ , $\kappa-\kappa/\Delta$	Difference between correlation for specific electrical conductivity, $\kappa$ , and hydrogen-ion concentration, $P_H$ , $\kappa-P_H$	Difference between correlation for ratio of conductivity to depression, $\kappa/\Delta$ , and hydrogen-ion concentration, $P_H$ , $\kappa/\Delta-P_H$
First series:							
Pima and Meade.....	1, 5	+0.1829	+0.4273	+0.4538	+0.2443	+0.2708	+0.0265
Do.....	3, 7	+0.0827	+0.2633	+0.5349	+0.1746	+0.4462	+0.2716
Do.....	1, 3, 5, 7	+0.1309	+0.3624	+0.4738	+0.2315	+0.3430	+0.1115
Pima and Acala.....	2, 4, 6	+0.1974	+0.2969	+0.6320	+0.0995	+0.4346	+0.3351
Hybrid and Pima.....	1, 5	+0.1577	+0.3783	+0.5672	+0.2206	+0.4095	+0.1889
Do.....	3, 7	+0.3755	+0.6550	+0.1408	+0.2794	+0.2347	+0.5142
Do.....	1, 3, 5, 7	+0.3266	+0.5241	+0.4307	+0.1975	+0.1041	+0.0934
Hybrid and Meade.....	1, 5	+0.2428	+0.4087	+0.6741	+0.1659	+0.4314	+0.2655
Do.....	3, 7	+0.2996	+0.4483	+0.3896	+0.1487	+0.0901	+0.0586
Do.....	1, 3, 5, 7	+0.2948	+0.4768	+0.5790	+0.1821	+0.2843	+0.1023
Second series:							
Pima and Meade.....	1, 5	+0.4010	+0.6545	+0.2074	+0.2535	+0.1936	+0.4470
Do.....	3, 7	+0.3772	+0.0917	+0.0462	+0.2855	+0.3310	+0.0455
Do.....	1, 3, 5, 7	+0.3638	+0.4033	+0.1692	+0.0395	+0.1946	+0.2341
Pima and Acala.....	2, 4, 6	+0.2273	+0.2282	+0.1170	+0.0009	+0.1103	+0.1112
Hybrid and Pima.....	1, 5	+0.1550	+0.6289	+0.1149	+0.4738	+0.0401	+0.5139
Do.....	3, 7	+0.3047	+0.1610	+0.0145	+0.1437	+0.3193	+0.1755
Do.....	1, 3, 5, 7	+0.2664	+0.4187	+0.0748	+0.2123	+0.1310	+0.3439
Hybrid and Meade.....	1, 5	+0.5040	+0.9494	+0.4942	+0.4554	+0.0098	+0.4552
Do.....	3, 7	+0.5162	+0.4995	+0.1615	+0.0166	+0.6776	+0.6610
Do.....	1, 3, 5, 7	+0.4866	+0.7072	+0.1545	+0.2265	+0.3262	+0.5527

For further evidence on this point we may turn to Table XXI, showing the correlation between the various constants in the first and second series.<sup>26</sup> The coefficients for the Upland and hybrid series have been shown to be so nearly zero that they may be disregarded for the moment. Considering the more substantial values of the correlation for the Egyptian cotton, it appears that the highest values found are those for  $\Delta$ , which are of the order 0.40. The correlations for  $\kappa$  and  $\kappa/\Delta$  are of the order 0.30. The correlations for  $P_H$  are sensibly zero.

These results seem to indicate that the absorption of electrolytes from the soil is not the most important, or at least the sole, factor in the differentiation of the plants. This is shown by the fact that the correlations for osmotic concentration are higher than those for electrical conductivity and for ratio of conductivity to freezing-point lowering. That synthesized solutes are of importance in differentiating the plants of the same duplet or triplet at the moment at which the collection is made, is shown by the substantial values of the correlations for hydrogen-ion concentration between the members of the same duplet or triplet, as demonstrated for the Egyptian and Upland plants in the seventh column of Table XIII and for the parental and the hybrid types in the seventh column of Table XVIII.

It is perhaps not surprising that the correlation for total solutes should be higher than that for either of its measured constituents.

<sup>26</sup> In view of the fact that we hope later to discuss in detail the problem of the relationship between the properties of the soil solution and those of the plant tissue fluids, it seems undesirable to consider the cross correlations between the first and second series of constants as set forth in Table XXII.

We may turn again to Table XXIV for a comparison of the magnitudes of the correlations for specific electrical conductivity, ratio of conductivity to freezing-point depression, and hydrogen-ion concentration.

In the first series the correlation for specific electrical conductivity is in every instance higher than that for the ratio of conductivity to freezing-point depression. In the second series the same relationship holds in only 7 of the 10 comparisons. The differences in both first and second series are generally low. We have no explanation of this result to suggest.

A comparison of the coefficients of correlation for hydrogen-ion concentration with that for specific electrical conductivity indicates that in the first series of determinations the correlation for conductivity is higher than that for hydrogen-ion concentration, whereas in the second series of determinations the reverse is true. This result is substantiated by a comparison of the coefficients for the ratio of conductivity to freezing-point depression with those for hydrogen-ion concentration.

The determination of the environmental (soil or atmospheric) or the internal conditions to which these differences are due presents a problem for future investigation.

#### SUMMARY AND CONCLUSIONS

While this paper is in substance a contribution to the comparative physiology of Egyptian and Upland cotton, its purpose has been broader. It is one of a series of investigations which have been undertaken with the conviction that the relative capacity for growth and production of different crop plants under special conditions ultimately depends upon intrinsic morphological and physiological differences, and that in our attempts to secure varieties of crop plants which may be successfully grown under particularly stringent environmental conditions (such as those of extremes of temperature, or of aridity of the soil or atmosphere, or of the concentration or reaction of the soil solution) we will in the long run make the most rapid and certain progress by determining the particular variables which fit the plant for growth under these conditions. With a working knowledge of these variables, based on investigations of native vegetations and of crop plants, it will be possible to select varieties which seem most suitable for growth under peculiar environmental conditions.

The present investigation has been limited to osmotic concentration, specific electrical conductivity and acidity in terms of hydrogen-ion concentration in the leaf-tissue fluids of Pima Egyptian and of Meade and Acala Upland cottons and of those of the  $F_1$  hybrid between Pima and Meade cotton as grown under irrigation in southern Arizona. This has been in part because the technique for work with these variables is in a more satisfactory state of development than that for others which may be of importance. The limitation has, however, been primarily because investigations on natural vegetation have indicated a close relationship between the first two of these variables and the dryness and salinity of the substratum.

The statistical constants for sap properties are based on a large number of determinations on plants grown in 1921 on subplots at the Cooperative Testing Station, Sacaton, Ariz. The determinations were made in two series, the first based on collections of tissue made from August 6 to August 16; the second on samples of tissue gathered from August 19 to

August 27. The results for the two series of determinations differ slightly but are mutually confirmatory, and substantiate those of preliminary determinations made in 1920. They establish the following differences between the tissue fluids of the Egyptian and the Upland type of cotton.

The osmotic concentration of the leaf-tissue fluids is higher in Egyptian than in Upland cotton. Thus, in the first series of determinations, the freezing-point depressions of the Egyptian plants grown from self-fertilized seed average 1.404 as compared with 1.353 in the associated Meade plants grown from self-fertilized seed; the Pima plants grown from bulk seed average 1.357 as compared with 1.352 in the associated Meade plants grown from bulk seed; the Pima plants grown from bulk seed average 1.340 as compared with 1.300 in the associated Acala plants grown from bulk seed. In the second series of determinations the average for Pima plants from self-fertilized seed is 1.298 as compared with 1.212 in the Meade plants from self-fertilized seed; the average for Pima plants from bulk seed is 1.257 as compared with 1.201 for Meade plants from bulk seed; the average for Pima plants grown in association with Acala is 1.280 as compared with 1.182 in the Acala. While the differences are not large, varying from less than 1 per cent to slightly more than 8 per cent, they are in the main far larger than their probable errors and are consistent throughout in indicating a higher osmotic concentration in the tissue fluids of the Egyptian cotton. The differences are greater in the second series than in the first.

While these differences are small as compared with those which have been demonstrated between the native plant species of humid and those of arid regions, they may be of significance in the growth of the plants. Critical experiments to determine whether this is the case still remain to be made.

The electrical conductivity of the leaf-tissue fluids of Egyptian cotton is significantly higher than that of either of the Upland cottons compared. This result is found consistently in each of the series investigated. The differences range from 2.93 to 3.77 per cent in the first series and from 8.19 to 9.22 per cent in the second series. The difference between the two cottons is greater in the collections made at the later date, notwithstanding the fact that the actual values of conductivity are lower.

The higher values of electrical conductivity indicate that the Pima Egyptian cotton is capable of taking up from the soil and retaining in solution in the tissue fluids larger quantities of conducting electrolytes than the Upland cottons considered.

The ratio of specific electrical conductivity,  $\kappa$ , to freezing-point depression,  $\Delta$ , is in general somewhat higher in Egyptian than in Upland cotton. The differences are not, however, large.

This result suggests that the tissue fluids of the Egyptian cotton contain relatively, as well as absolutely, larger quantities of solutes capable of carrying the electric current than those of Upland cotton.

The reaction of the expressed tissue fluids is acid in both types of cotton. The values of  $P_H$  are lower in Egyptian than in the Upland type. Thus acidity, as measured in terms of hydrogen-ion concentration, is greater in the Egyptian than in the Upland. In the first series of determinations the value of  $P_H$  is 5.245 in the Pima as compared with 5.346 in the associated Meade plants grown from self-fertilized seed; 5.269 in the Pima as compared with 5.396 in the associated plants grown from bulk

Meade seed; 5.257 in the Pima as compared with 5.302 in the associated Acala plants. In the second series of determinations the differences are of about the same order.

A comparison of the sap properties of the  $F_1$  hybrids between Pima and Meade ( $P \times M$ ) with those of the two parents gives the following results:

The osmotic concentration of the tissue fluids of the hybrid is lower than that of either of the parent types.

When the hybrid is compared with parental cultures grown from self-fertilized seed, the hybrid has an osmotic concentration 3.87 per cent lower than that of the Meade parent and 7.36 per cent lower than that of the Pima parent in the first series of determinations, and 6.18 per cent lower than that of the Meade parent and 12.4 per cent lower than that of the Pima parent in the second series of determinations.

When the hybrid is compared with the parental forms grown from bulk seed, the hybrid has an osmotic concentration 4.34 per cent lower than that of the Meade parent and 4.75 per cent lower than that of the Pima parent in the first series of determinations, and 8.18 per cent lower than that of the Meade parent and 12.3 per cent lower than that of the Pima parent in the second series of determinations.

Since the Upland cotton has a lower osmotic concentration than the Egyptian, the difference between the hybrid and the Upland type is of necessity smaller than that between the hybrid and the Egyptian type. The difference between the hybrid and the parent forms is greater in the samples taken later in the season.

The specific electrical conductivity of the hybrid is in all cases lower than that of either of the parent types. In the first series of determinations the conductivity of the hybrid tissue fluids was 4.35 per cent lower than that of the Meade parent and 7.84 per cent lower than that of the Pima parent when the two parents were grown from self-fertilized seed. The hybrid was 1.53 per cent lower than the Meade parent and 4.39 per cent lower than the Pima parent when the parent forms were grown from bulk seed. In the second series of determinations the percentage differences range from 0.33 per cent in comparison with the self-fertilized Meade parent to 9.11 per cent in comparison with the Pima parent from bulk seed.

In general, the differences between the parent and the hybrid forms are greater in the determinations made later in the season.

Since the conductivity is lower in the Upland than in the Egyptian type, the difference between the constant for the hybrid and that for the Upland parent form is smaller than that between the hybrid and the Egyptian parent type.

The ratio of specific electrical conductivity to freezing-point depression,  $\kappa/\Delta$ , is generally but not invariably higher in the hybrid than in the parent forms. In the first series of determinations the ratios are sometimes higher in the hybrid and sometimes higher in the parents. In the second (later) series, the hybrid gave a ratio from 3.7 to 7.1 per cent higher than the parent forms. The differences are, therefore, larger and more clearly significant in comparison with their probable errors in the later than in the earlier series of determinations.

The hydrogen-ion concentration of the hybrid is higher than that of the Egyptian parent, but lower than that of the Upland parent. Thus the hybrid tissue fluids are less acid than those of the Pima parent, but

more acid than those of the Meade parent. In short, the hybrid is intermediate in acidity between the two parental forms.

The results for hydrogen-ion concentration are of particular interest since in respect to this physical constant the behavior of the hybrid is quite different from that in respect to osmotic concentration and specific electrical conductivity, both of which show values for the hybrid which are distinctly lower than those of either of the parent forms.

Hydrogen-ion concentration is therefore in full agreement with the great majority of morphological characters (other than size) with respect to which the hybrid is intermediate between the two parent forms.

The foregoing statements summarize the results as far as a comparison of the two cottons and their  $F_1$  hybrid is concerned. We now have to consider certain outstanding features of these series of constants as a whole.

Conspicuous among these general features is the fact that practically all the constants differ in the two series of determinations, made earlier and later in the month of August.

Both osmotic concentration as measured by the depression of the freezing point,  $\Delta$ , and electrical conductivity,  $\kappa$ , are lower in the second series of determinations than in the first, in the case of both hybrids and parents. The differences are relatively large, and clearly significant. The ratio of specific electrical conductivity to freezing-point depression,  $\kappa/\Delta$ , is on the average lower in the second series of determinations made on the parent forms, but higher in the second series made on the hybrids. The difference between the first and second series of determinations made on the hybrids is much larger than that between the first and second series made on the parent forms. The values of  $P_H$ , measuring acidity in terms of hydrogen-ion concentration, are uniformly higher in the second than in the first series of determinations. Since higher values of  $P_H$  indicate lower acidities it is clear that the concentration of the hydrogen ion is also lower in the second series.

Thus the concentrations of total solutes (molecules and ions) of all ionized solutes and of the hydrogen ion are lower in the second than in the first series. The explanation of these changes must await further investigation.

In its bearing on the problem of the differentiation of the two types of cotton it is perhaps significant that the second series, while showing absolutely lower values of osmotic concentration, specific electrical conductivity, and concentration of the hydrogen ion, shows uniformly a greater differentiation with respect to these variables. Thus the differentiation between the two types increases with the advance of the season.

The direct and the cross correlations between the homologous samples of the first and second series of determinations have been discussed. It is shown that the relationships for the Egyptian is very different from that for the Upland and hybrid plants. The explanation of these differences must await further investigation.

There is a significant positive correlation between the members of the same duplet or triplet (the plants or groups of plants of the two or three types immediately associated at the same station of the field). This indicates a large influence of soil salinity, or some other factor of substratum heterogeneity, or an influence of variations in atmospheric conditions during the period throughout which the samples were taken.



In these correlations between the plants of the same duplet or triplet, the coefficients for osmotic concentration are higher than those for specific electrical conductivity, or for the ratio of specific electrical conductivity to freezing-point depression. They are in practically every instance higher than those for hydrogen-ion concentration. Thus the concentration of total solutes seems to be more influenced by the environmental conditions than the concentration of all electrolytes or of the hydrogen-ion.

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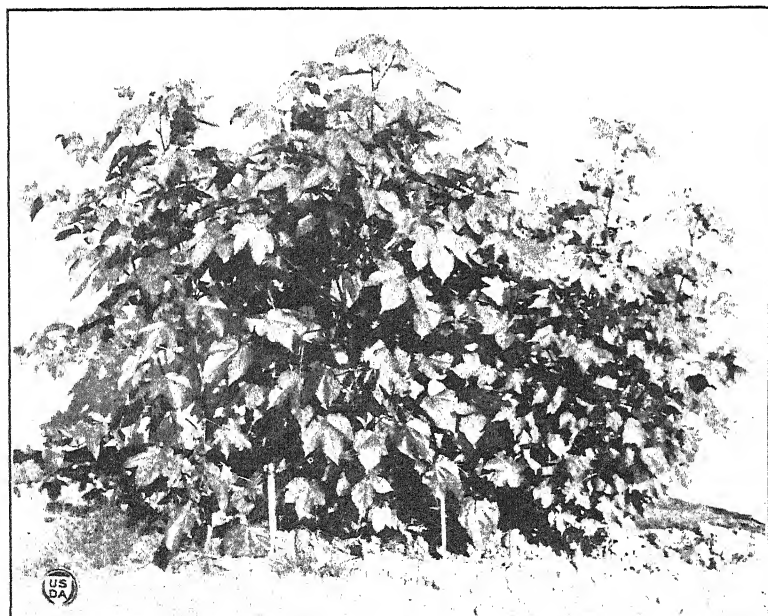
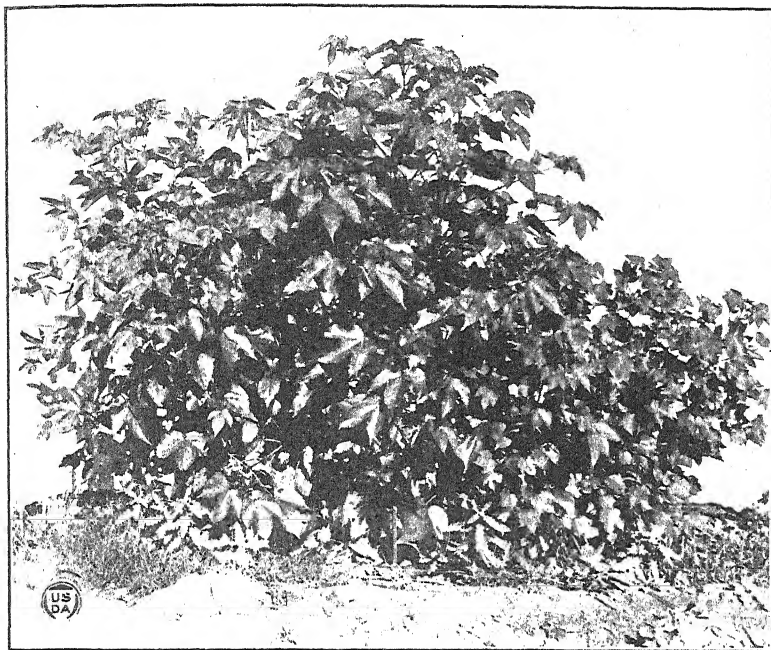
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PLATE 1

Typical triplets of Pima Egyptian, Meade Upland, and  $F_1$  hybrid between Pima and Meade cotton, showing in each figure a plant of Pima at the left and a plant of Meade at the right of the  $F_1$  hybrid, which occupies the center of the group.

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## SELECTIVE FERTILIZATION IN COTTON<sup>1</sup>

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### INTRODUCTION

It has been shown that in cotton, under natural conditions of pollination, a large majority of the ovules are self-fertilized, although the flower is well adapted to cross-pollination and foreign pollen usually reaches the stigmas (7,<sup>2</sup> p. 9, 10, 34, 35). There is also evidence of complete compatibility, even between different species such as Upland and Egyptian cottons, for pollen of both species proved to be equally effective in accomplishing fertilization when applied separately to the stigmas of Egyptian cotton flowers (7, p. 40-42). The question therefore suggests itself whether selective fertilization, in favor of the like pollen when unlike pollen also is present, is a factor in the observed predominance of self-fertilization.

The results of experiments by Balls, by McLachlan and by Kearney, summarized in another paper (7, p. 42-49) showed that selective fertilization does occur in cotton. When the stigmas of Egyptian cotton were pollinated simultaneously with approximately equal quantities of Egyptian and of Upland pollen, a large majority of the ovules were found to have been fertilized by the like pollen. The percentage of Egyptian  $\times$  Upland hybrids resulting from such double pollinations was  $10.8 \pm 0.6$  in an experiment performed by Argyle McLachlan and  $18.4 \pm 1.5$  in an experiment conducted by one of the writers, whereas, if both pollens had been equally effective in accomplishing fertilization, 50 per cent of the resulting plants should have been hybrids.

Yet no general conclusion could be drawn as to the importance of selective fertilization in cotton, because experiments made hitherto in Arizona have not afforded decisive evidence that it occurs in the Upland type also. Reciprocal double-pollinations on Upland cotton by McLachlan indicated little or no selective fertilization, the percentage of Upland  $\times$  Egyptian hybrids having been  $42.3 \pm 1.1$ . Earlier experiments at Sacaton, Arizona, to determine whether selective fertilization takes place in Upland cotton failed because nearly all of the artificially pollinated flowers on the Upland plants were lost by abscission.

The writers, assisted by Max Willett and Dow D. Porter, have now succeeded in obtaining conclusive evidence that selective fertilization, in favor of the like pollen, takes place in Upland, as well as in Egyptian cotton. The purpose of this paper is to describe the experiments which yielded this evidence.

<sup>1</sup> Accepted for publication Dec. 14, 1923.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 340.

Selective fertilization, in favor of like as compared with unlike, although compatible pollen when both are present on the stigmas, has been shown by Jones to occur in maize and tomato (3, 4, 5, 6). Experiments of Heribert-Nilsson indicated that it takes place also in *Oenothera* (2). The fact that members of such distantly related families as Poaceae, Malvaceae, Onagraceae, and Solanaceae manifest selective fertilization in favor of the like pollen, points to the conclusion that the phenomenon is by no means uncommon among flowering plants. Its probable importance as a factor contributing to the differentiation of plant forms has been discussed by Jones (5).

The evidence for selective fertilization brought forward by Jones, supplemented by data given in this paper, applies only to cases when "germ cells from two individuals of different type are presented at the same time in excess so that not all can fulfill their function" (4, p. 253), and has no bearing upon the question whether there is selective fertilization among the several gametes produced by the same individual.

### METHOD OF INVESTIGATION

The experiments were conducted at the Cooperative Testing Station at Sacaton, Arizona. The pollinations were made in 1922. The flowers to be pollinated were emasculated in the evening preceding anthesis and were enclosed in paper bags to prevent accidental cross-pollination. The flowers which were to supply the pollen were bagged at the same time. The pollinations were made at 1 p. m. of the following day, the procedure having been to detach the flower supplying the pollen and brush the stigmas of the emasculated flower lightly with the clustered anthers of the staminate flower (7, *Pl. I, IV, V*).

Because of the mechanical difficulty of mixing the two kinds of pollen in equal proportion, recourse was had to the method of double pollination used in an earlier experiment (7, p. 46). This consisted in pollinating one-half of the flowers first with the like pollen and then with the unlike pollen and reversing the order of application on an equal number of flowers. The two pollens were applied in as nearly as possible equal quantity and the interval between the two pollinations was negligible.

Two experiments were performed, the pollinations of the first experiment having been made during the period July 21 to 30 and those of the second experiment August 10 to 13. The Egyptian type of cotton was represented in both experiments by the Pima variety. The Upland type was represented in the first experiment by the Lone Star variety and in the second experiment by the Acala variety. The stocks of each variety used in the experiments were not inbred families but had been grown from seed produced under conditions of isolation and were judged to be varietally pure. Well grown plants were used in both experiments, those in experiment 1 having been located in adjacent plats, and those in experiment 2 in the same plat. Two hundred flowers of each variety were pollinated in each experiment.

The seeds produced by the double-pollinated flowers were planted in 1923. As many more bolls matured from the double-pollinated flowers on the Pima than on the Upland plants, the former yielded much greater quantities of seed than the latter.<sup>3</sup> The seeds from each lot of Pima

<sup>3</sup> Abscission of the young bolls is always more pronounced in Upland than in Pima cotton as grown Sacaton.



flowers were therefore mixed thoroughly and a representative sample of each lot was planted. The quantities of seed from the several pollinations of the Upland flowers were so small that all were planted.

The seeds representing each sequence of pollination (like + unlike and unlike + like) on each type of cotton in each experiment were planted as separate populations and these populations were further subdivided, seeds from the upper and the lower halves of the bolls having been planted separately. This gave a total of eight ultimate populations from each of the two experiments, as follows:

Pima $\times$ (Pima + Upland).....	Upper halves of bolls.
Do.....	Lower halves of bolls.
Pima $\times$ (Upland + Pima).....	Upper halves of bolls.
Do.....	Lower halves of bolls.
Upland $\times$ (Upland + Pima).....	Upper halves of bolls.
Do.....	Lower halves of bolls.
Upland $\times$ (Pima + Upland).....	Upper halves of bolls.
Do.....	Lower halves of bolls.

These ultimate populations were variously combined in considering the data from different points of view. The plants grown from upper and lower seeds of the bolls from each pollination are treated as separate populations only under the heading "Rate of growth of the pollen tubes in relation to selective fertilization."

Four seeds were planted to the hill and no thinning was done. A total of 2,349 plants was obtained from the double-pollinated flowers of Pima and a total of 1,419 plants from the double-pollinated flowers of the Upland varieties. The difference in number of plants was due to the quantities of seed of the two types available for planting and not to better survival in the germinating and seedling stages of the plants from seeds produced by Pima flowers. For both types of cotton the number of plants which survived averaged 2.6 per hill.

On July 19 and 20, when the plants had developed sufficiently to make recognition of the hybrids unquestionable,<sup>4</sup> counts were made of the total number of plants and of  $F_1$  hybrids in each population and from these data the percentages of hybrids were computed.

#### COMPARISON OF EGYPTIAN AND UPLAND COTTONS AS TO SELECTIVE FERTILIZATION

The data from the two experiments, showing the evidence for selective fertilization in both types of cotton, are presented in Table I. In each case the results from pollination first with like and then with unlike pollen, and the converse, are combined. It will be shown that the pollen applied first fertilized a greater number of ovules than the pollen applied afterward to the same stigmas, but it seems reasonable to assume that the percentage of hybrids obtained by combining the populations from like + unlike and unlike + like pollinations as one array corresponds with what would have been obtained had it been practicable to mix the two pollens in equal quantity before application to the stigmas.<sup>5</sup>

<sup>4</sup> The characters distinguishing  $F_1$  hybrids of Pima and Upland cotton from the parental types were described in another paper (8, p. 6, 7).

<sup>5</sup> Nevertheless, a small error is involved owing to the fact that populations of unequal size were obtained from the pollinations like + unlike and unlike + like. If, however, the percentages of hybrids for each sequence of pollination (as given in Table II) are averaged for each variety in each experiment, the averages depart but slightly from the percentages based upon the two sequences taken as one array, as given in Table I, and the significance of the results is not altered.

TABLE I.—Percentages of  $F_1$  hybrids resulting from pollination of Egyptian (Pima) and of Upland (Lone Star and Acala) cotton flowers with approximately equal quantities of Egyptian and Upland pollen

Populations from double pollinations of:	Number of—		Percentage of hybrids.
	Plants.	Hybrids.	
EXPERIMENT 1			
Pima with Pima+Lone Star and Lone Star+Pima...	1, 381	442	32. 0 ± 0. 85
Lone Star with Lone Star+Pima and Pima+Lone Star	752	166	22. 1 ± 1. 02
EXPERIMENT 2			
Pima with Pima+Acala and Acala+Pima.....	968	163	16. 8 ± 0. 81
Acala with Acala+Pima and Pima+Acala.....	667	220	33. 0 ± 1. 23
BOTH EXPERIMENTS AS ONE ARRAY			
Double pollinations on Egyptian.....	2, 349	605	25. 8 ± 0. 61
Double pollinations on Upland .....	1, 419	386	27. 2 ± 0. 80

Considering as one array all plants resulting from double-pollinations of the Egyptian (Pima) flowers and of the Upland flowers, respectively, (Table I, bottom section) it is evident that the percentages of hybrids were practically the same, the difference having amounted to only  $1.4 \pm 1.0$  per cent. In both cases approximately three-quarters of the ovules were fertilized by pollen of the same type and the departures from the 50 per cent expected if there had been no selective fertilization are extremely significant.

It is interesting that while in the first experiment the Pima flowers yielded a significantly higher percentage of hybrids than the Upland flowers (difference  $9.9 \pm 1.33$ ), in the second experiment it was the Upland flowers that yielded a significantly higher percentage of hybrids (difference  $16.2 \pm 1.47$ ). The cause of this difference in the results of the two experiments may only be conjectured. Differences in the relative viability of the several pollens would seem to offer the most plausible explanation, but such data as were obtained show practically equal viability.

Pollen from five flowers each of Pima and of Lone Star, each flower having been borne by a different plant, was tested as to its ejection when immersed in a 5 per cent solution of cane-sugar (7, p. 22-25). The estimated percentage of grains which ejected was 90 per cent for each of the Pima flowers and ranged from 80 to 90 per cent, with an average of 87 per cent, for the five flowers of Lone Star. The tests were conducted during the period when the double pollinations of experiment 1 were being made. Similar tests of pollen from four flowers of Acala cotton made at the outset of experiment 2, showed ejection of from 90 to 95 per cent of the grains in the field of the microscope.

#### SEQUENCE OF APPLICATION OF THE TWO POLLENS AS AFFECTING THE PERCENTAGE OF HYBRIDS

Higher percentages of hybrids were obtained when the unlike pollen was applied first than when the like pollen was applied first. The data given in Table II show that this was the case for both types of cotton in

both experiments, although the differences were significant in only two of the four comparisons, that of the Pima flowers in experiment 1 and that of the Upland (Acala) flowers in experiment 2. The differences in these cases were, respectively, 6.3 and 8.4 times the probable error of the difference. The probable explanation is that in some of the flowers the surface of the stigmas was so well covered by the pollen applied first that many of the grains of the other kind of pollen did not come into contact with the stigmatic surface and hence were unable to effect fertilization. It should be noted that the highest percentage of hybrids resulting from application of the unlike pollen first (Acala with Pima + Acala in experiment 2) is significantly lower than the 50 per cent expected if there had been no selective fertilization, the departure having been  $7.8 \pm 1.76$ .

TABLE II.—Percentages of hybrids resulting from double pollination with (a) the like pollen applied first, (b) the unlike pollen applied first

Sequence of application of the like and unlike pollens.	Number of—		Percentage of hybrids.
	Plants.	Hybrids.	
EXPERIMENT I			
(a) Pima with Pima+Lone Star.....	741	200	27.0 $\pm$ 1.10
(b) Pima with Lone Star+Pima.....	640	242	37.8 $\pm$ 1.29
Difference in favor of unlike pollen applied first.....			10.8 $\pm$ 1.70
(a) Lone Star with Lone Star+Pima.....	481	102	21.2 $\pm$ 1.26
(b) Lone Star with Pima+Lone Star.....	271	64	23.6 $\pm$ 1.74
Difference in favor of unlike pollen applied first.....			2.4 $\pm$ 2.15
EXPERIMENT 2			
(a) Pima with Pima+Acala.....	627	100	15.9 $\pm$ 0.98
(b) Pima with Acala+Pima.....	341	63	18.5 $\pm$ 1.42
Difference in favor of unlike pollen applied first.....			2.6 $\pm$ 1.73
(a) Acala with Acala+Pima.....	300	69	22.3 $\pm$ 1.59
(b) Acala with Pima+Acala.....	358	151	42.2 $\pm$ 1.76
Difference in favor of unlike pollen applied first.....			19.9 $\pm$ 2.37

#### SELECTIVE SURVIVAL AS A FACTOR AFFECTING THE PERCENTAGES OF HYBRIDS

The percentage of hybrids in an adult population from double-pollinated flowers would not represent the actual degree of selective fertilization if there had been selective survival in favor of either the homozygotes or the heterozygotes. It is possible, even, that what would appear to be a case of selective fertilization in favor of like pollen might be, in reality, merely a result of pronounced selective survival of the homozygotes. It is important, therefore, to examine critically the evidence as to whether there had been selective survival in the experiments under discussion.

Whether selective survival took place during germination and in the early stages of seedling growth is the first question to be examined. Had this been the case it seems probable that the heterozygotes rather than the homozygotes would have benefitted, since the Pima-Upland hybrid plants soon become conspicuously larger and more vigorous than the Pima or the Upland plants resulting from fertilization by like pollen. If there

had been selective survival of the heterozygous seeds or seedlings, the percentage of hybrids in the adult population would have been higher than the percentage of ovules fertilized by the unlike pollen. In that case the data in Table I would indicate less than the full measure of selective fertilization in favor of the like pollen.

Four seeds were planted in each hill but in many of the hills only one or two plants survived. If there had been selective survival in favor of either the homozygotes or the heterozygotes, it seems probable that the percentage of hybrids obtained where conditions were relatively unfavorable (hills containing one or two plants) would differ from the percentage obtained where conditions were more favorable (hills containing three or four plants). The percentages of hybrids under both conditions were therefore computed for the populations resulting from the double-pollination of Pima and of Upland flowers, respectively, and are given in Table III.

TABLE III.—Percentages of hybrids in hills containing 1 or 2 and in hills containing 3 or 4 plants, respectively, four seeds having been planted in each hill

Number of plants per hill.	Percentages of hybrids in populations resulting from double pollination of—			
	Pima flowers.		Upland flowers.	
	Total plants.	Percentage of hybrids.	Total plants.	Percentage of hybrids.
1 or 2.....	676	23.6 ± 1.10	411	29.6 ± 1.52
3 or 4.....	1,657	26.6 ± 0.73	998	26.3 ± 0.94
Difference.....		3.0 ± 1.32		3.3 ± 1.79

The data presented in Table III show that in the population from double-pollinated Pima flowers the percentage of hybrids was greater where the conditions were more favorable to survival, while in the population from double-pollinated Upland flowers the converse was true. In neither population, however, was the difference significant, and it may be concluded that the percentages of hybrids obtained in these experiments were not appreciably affected by conditions operating after the seeds were planted.

The evidence seems conclusive that the case under consideration can not be accounted for as one of selective survival in the germinating and seedling stages. It remains to consider whether selective survival may have occurred at the time of fertilization or immediately thereafter. Since in the adult population homozygotes were much more numerous than heterozygotes, it might be assumed that even if equal numbers of the female gametes had been reached by both kinds of male gametes, an undue proportion of those reached by the unlike male gametes had perished because fertilization was not completed or because the resulting zygotes were unable to develop. Direct evidence on this point would be very difficult to obtain but the following considerations make it improbable that selective fertilization in this material can be explained in this way.

The mean number of ovules in the ovary of Pima cotton, as determined in 1922, was  $21.5 \pm 0.10$ . The mean number of seeds in the bolls which

developed from the double-pollinated Pima flowers in the experiments here described was  $16.8 \pm 0.14$ . Therefore, an average of 4.7 ovules had failed to develop into seeds. The proportion of hybrids in the adult population from seeds borne by Pima plants, taking the populations of the two experiments as one array, was 25.8 per cent (Table I). It is computed therefore that the mean number of heterozygous seeds per boll was 4.33 (25.8 per cent of 16.8). Assuming that all of the ovules which failed to develop represent heterozygotes or uncompleted heterozygous unions and adding the mean number of undeveloped ovules to the mean number of heterozygous seeds, a total of 9.03 is obtained as representing the mean number of possible heterozygotes per boll. Even this number is only 42 per cent of the mean number of ovules. A similar computation in regard to the Upland varieties in these experiments is impracticable because satisfactory data as to their mean numbers of ovules are not available.

There is, however, no reason to assume that all or most of the undeveloped ovules represent unsuccessful unions or attempts at union with unlike male gametes. The mean number of seeds per boll from the double-pollinated Pima flowers of these experiments (16.8) exceeds the average (16.5), for 10 lots of bolls from naturally pollinated flowers of the same variety, as given in another publication (7, p. 51, Table 30). The highest mean number for any of these lots was 18.6 or 2.9 fewer than the mean number of ovules. It is evident that under the most favorable conditions a number of the ovules fail to develop, probably because they are defective or because they are not reached by pollen tubes. The conclusions seem warranted, therefore, that the percentage of undeveloped ovules in the present experiments was not abnormally high and that there had been no selective survival at fertilization or in the early stages of development of the zygote.

#### RATE OF GROWTH OF THE POLLEN TUBES IN RELATION TO SELECTIVE FERTILIZATION

In seeking an explanation of selective fertilization, the possibility of a difference in the rapidity of germination and of pollen-tube development of the like and unlike pollens is the first point to be considered. An experiment was described in another paper (7, p. 42) in which it was sought, by excising the stigmas and style at successive intervals of time after pollination, and by comparing the degrees of fertilization thus attained, to determine the relative rates of growth of Pima and of Upland pollen deposited separately on the stigmas of different Pima flowers. The growth rates of the like and unlike pollens, under these conditions, were found to be very similar. The experiment was repeated in 1923, a number of Pima flower buds having been emasculated the evening before anthesis and pollinated at 8 a. m. the next day, some of the flowers with Pima pollen and others with Upland pollen (Acala variety). The styles of equal numbers of flowers of each pollination were then severed at the summit of the ovary at intervals of 8, 10, and 12 hours after the pollen was deposited.<sup>6</sup> A record was kept of the number of bolls which developed from each lot of flowers and of the number of seeds in each boll. The results are summarized in Table IV.

<sup>6</sup> This method was used by Heribert-Nilsson (2) in an investigation of the rate of pollen-tube growth in *Oenothera*. His results showed a more rapid growth of the like pollen.

TABLE IV.—*Relative rapidity of penetration of the ovary of Pima cotton by Pima and by Upland pollen tubes, as measured by the degrees of fertilization attained when the pistils were excised at successive intervals*

Number of hours from pollination to excision of the pistils.	Pollination with—					
	Pima pollen.			Upland pollen.		
	Flowers treated.	Percentage of bolls developed.	Mean number of seeds per boll.	Flowers treated.	Percentage of bolls developed.	Mean number of seeds per boll.
8.....	100	0	0	100	0	0
10.....	100	2. $\pm$ 0.94	8.5 $\pm$ 3.58	100	8. $\pm$ 1.83	11.5 $\pm$ 1.42
12.....	100	10. $\pm$ 2.02	5.3 $\pm$ 1.06	100	18. $\pm$ 2.59	7.8 $\pm$ 0.90

In Pima cotton, under normal conditions of pollination, from 75 to 97 per cent of the flowers develop bolls (7, *Table 34*, p. 55) and the bolls contain an average of from 14 to 18.5 seeds (7, *Table 30*, p. 51). Comparing with these figures the percentages of bolls developed and the mean numbers of seeds per boll as given in Table IV, it is evident that relatively few pollen tubes had penetrated the ovaries within 12 hours after deposition of the pollen. Yet, it should be noted that of the flowers excised at this time, one, pollinated with Pima pollen, developed a boll containing 18 seeds and one, pollinated with Upland pollen, developed a boll containing 20 seeds.

The data, so far from showing more rapid development of the like pollen, seem to indicate that better fertilization was attained by the unlike pollen when the time available for penetration of the ovary was shortened by excision of the pistil. In fact, however, the differences between the pollinations with Pima and with Upland pollen, in percentages of bolls and in mean number of seeds, for either the 10-hour or the 12-hour period, are in all cases less than three times the probable error of the difference.

The results of this experiment, agreeing in the main with those of a similar experiment previously reported, make it reasonably certain that, when separately applied, like pollen has no advantage over unlike pollen in its rate of penetration of the ovary of Pima cotton. It does not follow, however, that there may not be a difference in the rate of penetration when both pollens are present on the stigmas of the same flower. Jones (6) obtained evidence that in maize, when a mixture of two pollens was applied to the stigmas, there resulted a greater proportion of cross-fertilization in the upper than in the lower half of the ear, from which he inferred a slower rate of development of the unlike pollen. The indicated differences in rate of growth seem, however, too small to account for the degrees of selective fertilization shown in some of Jones' combinations.

Maize, with its elongated female inflorescence and extraordinarily long styles, is far superior to cotton as a subject for the study of differential fertilization. In Pima cotton the stigmas and styles average only 30 to 35 mm. in length and in the Upland varieties used in these experiments they average not more than two-thirds as long as in Pima. The ovary of both types is only 5 or 6 mm. long at anthesis. In view of these facts it seemed unlikely that evidence of differential fertiliza-

tion could be obtained with this material, but it was thought advisable to test the possibility by experiment.

In comparing cotton with maize in respect to differential fertilization, the distinction applies which was made by Jones in comparing the results of Correns with *Melandrium* and his own results with maize. "In the former the pollen tubes enter the ovary at a common point and are free to fertilize the first ovules they reach. The tubes which grow fastest, therefore, fertilize the ovules in the upper part of the ovary, leaving the slower-growing tubes to pass on down to the lower part. In *Zea* each ovule has a separate style, so that the longer the distance to traverse is, the less chance will the slower-growing tubes have of reaching the goal first" (6, p. 173). Since cotton resembles *Melandrium* rather than *Zea* in these points of structure, we should expect to find a greater proportion of cross-fertilized ovules in the lower than in the upper part of the ovary, if the tubes of the like pollen grow more rapidly than the unlike pollen tubes.

All bolls resulting from the double-pollination of Pima and Upland flowers in the two experiments described in the first part of this paper were halved when ripe and the seeds from the upper and from the lower halves were planted separately. (See p. 331.) The per centages of hybrids from upper and from lower seeds are stated in Table V, the populations from pollination with like+unlike and with unlike+like pollen having been taken as one array for each type of cotton in each experiment.

TABLE V.—Percentages of hybrids in populations grown from seeds from the upper and from the lower halves of bolls produced by double-pollinated flowers of Pima and of Upland cotton

Experiment and pollination.	Half of boll furnishing seed for planting.	Number of—		Percentage of hybrids.
		Plants.	Hybrids.	
EXPERIMENT 1				
Pima with Pima and Lone Star..	Upper.....	700	227	32.4±1.19
Do.....	Lower.....	681	215	31.6±1.20
	Difference...			0.8±1.69
EXPERIMENT 2				
Pima with Pima and Acala.....	Upper.....	405	63	15.6±1.21
Do.....	Lower.....	563	100	17.8±1.09
	Difference...			2.2±1.63
EXPERIMENT 1				
Lone Star with Lone Star and Pima.	Upper.....	373	86	23.0±1.47
Do.....	Lower.....	379	80	21.1±1.41
	Difference...			1.9±2.04
EXPERIMENT 2				
Acala with Acala and Pima.....	Upper.....	384	123	32.0±1.60
Do.....	Lower.....	283	97	34.2±1.90
	Difference...			2.2±2.48

The data given in Table V show in no case a significantly greater percentage of hybrids from seeds contained in the lower halves of the bolls than from seeds contained in the upper halves, and therefore afford no evidence that the rate of growth of the tubes of the unlike pollen is inferior to that of the like pollen when the two kinds of pollen are in direct competition.

The pistils of the Upland varieties are much shorter than the Pima pistils, but the Upland pollen fertilized equally well the ovules in the lower and in the upper half of the Pima ovary. Hence there is no evidence of a correlation between the length of the pistil and the length attainable by the pollen tubes, such as has been observed in other plants.<sup>7</sup>

#### DISCUSSION

Evidence has been presented by one of the writers that a large majority of the ovules normally are self-fertilized in both the Egyptian and the Upland type of cotton (7, p. 9, 10). It was also shown that pronounced selective fertilization occurs in the Egyptian type, but no conclusion could be drawn as to the general importance of this phenomenon as a factor in the observed preponderance of self-fertilization, owing to lack of conclusive evidence that selective fertilization takes place in the Upland type also (7, p. 42-49, 64). The results of experiments described in the present paper make it clear that selective fertilization occurs in both types and in about the same degree. Nearly 75 per cent of the ovules, on the average, are fertilized by pollen of the same type and variety when pollen of the other type is present on the stigmas simultaneously and in approximately equal quantity.

This evidence of pronounced selective fertilization should be considered in connection with the evidence given in another paper (7, p. 34, 35, 63) that as a rule a majority of the pollen grains reaching the cotton stigmas have originated in the same flower, in other words that there is usually present on the stigmas an excess of self-pollen over foreign pollen.<sup>8</sup> These findings afford a satisfactory explanation of the fact that self-fertilization predominates in cotton although the flower is so admirably adapted to cross-pollination (7, p. 12).

Egyptian cotton, although apparently not referable to any one of the species of *Gossypium* recognized by taxonomists, is very closely related to *G. barbadense* L. and to *G. peruvianum* Cav. and exhibits differences from Upland cotton (*G. hirsutum* L.) which are of specific if not subgeneric magnitude (8, p. 4-6, Plates I, III, V-XI). Nevertheless, the results of experiments described in another paper (7, p. 40-42) have shown that when Upland pollen alone was applied to the stigmas of Pima flowers the fertilization attained was at least equal to that resulting from the application of Pima pollen alone. It was found also that the reciprocal pollinations on Upland showed only a slight

<sup>7</sup> McClelland (9) ascertained that when two species of *Vanilla*, one of which had a relatively short and the other a long column, were cross-pollinated, pollen of the first type fertilized few or none of the ovules near the base of the ovary of the second type, while pollen of the second type (long column) fertilized the basal ovules of the first type (short column) even more readily than did its own pollen. On the other hand, Tokugawa found that when two species of *Lilium*, one having a long style and the other a short style, were reciprocally cross-pollinated, the pollen tubes of either species grew faster in the pistil of the same species than did the foreign pollen tubes (10, p. 29, Table XII).

<sup>8</sup> The seeming discrepancy that under natural conditions more of the ovules appear to be self-fertilized in Egyptian than in Upland cotton, although selective fertilization occurs in both types in practically the same degree, is removed by the observations previously recorded (7, p. 34-36) that the Upland flowers usually receive foreign pollen in greater quantity than the Egyptian flowers.



and possibly not a significant advantage in favor of the like pollen. The two kinds of pollen, therefore, do not differ appreciably in compatibility.

Tests made in sugar solution showed no important differences in the viability of the several pollens, and the very fact that the Egyptian and the Upland flowers, when pollinated with a mixture of pollen of the two types, yielded approximately equal mean percentages of hybrids indicates that the average viability of the two kinds of pollen was practically the same.<sup>9</sup>

The case of selective fertilization here described is clearly not attributable to differences in the viability or in the compatibility of the two kinds of pollen. The evidence is against the assumption that the phenomenon is one of selective survival of the homozygotes, at any stage from the formation of the zygote to the attainment of the adult state. So far as could be ascertained, the unlike pollen is not inferior to the like pollen in the rate of development of its tubes and in ability to unite with the female gametes. How, then, is the fact of selective fertilization in cotton to be explained?

The only hypothesis which seems to fit the observed facts is one previously advanced (7, p. 48) that the presence of like pollen in some way prevents the germination or subsequent development of many of the unlike pollen grains when both kinds are present on the stigmas. That the inhibiting factor does not reside in the stigmas themselves when like pollen is absent seems clear from the fact that when applied separately the unlike pollen is not inferior to the like pollen in rapidity of development and ability to effect fertilization.<sup>10</sup>

It is conceivable, however, that the presence of pollen of the same type may induce a physiological reaction in the stigmas which makes them a relatively unfavorable medium for the germination or growth of pollen of a different type. The further assumption must be made that, in spite of this unfavorable condition, some of the unlike pollen grains are able to accomplish fertilization, possibly because they are more resistant, possibly because they happen to be so placed as to avoid the tracts of stigmatic tissue affected by contact with the like pollen. It would seem that such of the unlike pollen grains as succeed in avoiding or overcoming this obstacle develop their tubes as rapidly as do the pollen grains of the same type, and that there is no appreciable difference in the readiness with which the two kinds of male gametes unite with the female gametes.

The double-pollination experiments with maize carried out by Jones involved pairs of types which showed various degrees of genetic difference, some being nearly and others very remotely related. A pronounced positive correlation was found to exist between the degree of selective fertilization in favor of like pollen and the genetic distinctness of the types, as indicated by the degree of heterosis manifested by the crosses between them. As Jones expresses it, "the handicap placed upon the foreign pollen is proportional to the germinal unlikeness" (4, p. 283). The experiments with cotton described in this paper involve only unrelated types which differ profoundly in their morphological characters.

<sup>9</sup> It is, of course, possible that, even with a difference in the pollen viability of two varieties, equal percentages of hybrids might result from reciprocal double-pollination if selective fertilization in favor of the like pollen were more pronounced in the variety having the less viable pollen.

<sup>10</sup> This fact is also difficult to reconcile with the suggestion made by Jones (4, p. 284, 285) that we are concerned here with a phenomenon analogous to the toxicity of foreign proteins.

Very pronounced heterosis is shown by hybrids between these types, yet the degree of selective fertilization in this case was much smaller than in some of the maize combinations tested by Jones, in which, when double-pollinated, there was a near approach to cross-sterility. It remains to be ascertained whether *Gossypium* is analogous to *Zea* in showing a smaller degree of selective fertilization between more nearly related forms.<sup>11</sup>

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<sup>11</sup> The results of experiments made by Balls (*l. p.* 122-125) indicate that the analogy exists, for he found that when either Egyptian or Upland cotton was pollinated with a mixture of pollen of the same type and pollen from Egyptian  $\times$  Upland  $F_1$  plants, the percentage of hybrids resulting was much higher than when a mixture of Egyptian and Upland pollens was applied to the stigmas. This is in conformity with Jones' findings in *Zea*, since the first generation hybrid is, of course, more nearly related to either parent than is the other parent.

# THE EFFECT OF FERTILIZERS ON THE DEVELOPMENT OF STEM RUST OF WHEAT<sup>1</sup>

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## INTRODUCTION

For many years there has been an opinion that the severity of rust attacks on wheat and other cereals was influenced profoundly by available soil nutrients. Especially was it thought that heavy fertilization with nitrogenous manures was conducive to abundant development of rusts. Most farmers, and many scientists, did not distinguish between the different kinds of rusts, so that many of the early observations have but little value. About ten or twelve years ago, however, many farmers in Minnesota and neighboring States asserted that the kind of soil on which wheat was grown, as well as the amount of barnyard manure applied, affected very greatly the development of stem rust, *Puccinia graminis* Pers. In some localities there also was a general impression that wheat grown on clover soil was very likely to be injured badly by the rust. These observations seemed to indicate that nitrogenous fertilizers predisposed wheat to attack by *Puccinia graminis*, and the results of controlled experiments made by several European investigators apparently supported these views.

Comes (7)<sup>3</sup>, Spinks (30), Hiltner (12), Stranak (33) and others published the results of experiments which indicated that soil fertilization influenced profoundly the severity of stripe rust, *Puccinia glumarum* (Schm.) Erikss. and Henn., on wheat. Experiments conducted by the senior author (31) show that in general the absence or presence, in excessive amounts, of various nutrient substances, such as nitrogen and phosphorus salts, did not directly affect the immunity or susceptibility of wheats to *P. graminis*. Conditions favoring normal host development increased the vigor of the rust. It seemed desirable to make more extensive experiments in the field, on different types of soil, and over a period of several years. Empirical experiments of a practical nature therefore were begun in 1914 and were continued for eight years. The writers tried to ascertain facts, the explanation of which was sought in special investigations.<sup>4</sup>

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<sup>3</sup> Reference is made by number (italic) to "Literature Cited," p. 377.

<sup>4</sup> Dr. Freeman Weiss and Dr. C. R. Hursh made these studies as a part of the cooperative project between the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Agriculture of the University of Minnesota. The results will be published in separate papers.

## REVIEW OF PREVIOUS WORK

A summary of what has been published on the factors which predispose to or protect plants from disease would comprise many bulky volumes. It should be axiomatic that different host plants and pathogenes are affected differently by environmental conditions. It is difficult or impossible, therefore, to establish universally applicable principles, and, for this reason, only the most relevant literature is reviewed in this paper.

Little (15), in 1883, stated that low, rich soils and heavy manuring, especially with nitrogenous manures, predisposed wheat to rust. Anderson (1), in 1890, and Bolley (5), in 1889, expressed a similar opinion. Anderson found hard, flinty wheats resistant to rust. He thought resistance due possibly to the large amount of silica in such plants, and advised against the use of excessive quantities of nitrogenous fertilizers. He recommends that salt, iron sulphate, and lime be used to prevent rust.

Petermann (22, p. 15-16) reported in 1902 that wheat manured with superphosphate was very badly rusted, while that fertilized with volcanic slag was almost free. He thought the difference in resistance was due to differences in the strength of the cell walls.

In 1903, Ward (38), showed lack of minerals did not confer immunity from *Puccinia dispersa* Erikss. on bromes.

Remer (24) observed in 1903 the effect of fertilization on cereal rusts in Silesia. He concluded that even a slight excess of nitrogen predisposed cereals to rust. Stable manure applied to soil previously devoted to clover predisposed wheat and oats especially. He thought that excessive vegetative vigor, consequent lodging, and greater leaf surface favored the development of rust, and that acid phosphate usually inhibited its development on plants grown in soils in which there was not too much nitrogen. Potash did not predispose his cultures to rust.

McAlpine (16), in 1906, found that the most vigorous plants often were most severely rusted and that early maturity favored escape from rust injury. Johnson (14), in 1911, stated that wet soils and luxuriant growth predisposed timothy to the timothy rust, *P. graminis phleipratisensis* Erikss. and Henn. Montemartini (20) stated that starved plants were immune from rust and that phosphates always increased resistance. Freeman and Johnson (8) cite general observations on the apparent predisposing effect of nitrogenous fertilizers.

Biffen (4), in 1912, found *P. glumarum* most abundant on plants which had received a complete fertilizer. The amount of rust decreased with a decrease in the amount of fertilizer used.

Comes (7) states that nitrogen predisposed plants to rust, while non-nitrogenous fertilizers, especially potash, tended to increase resistance. He found that the concentration of organic acids was lowered, and that resistance to infection was proportionately lowered.

Spinks (30) studied the effect of nutrient salts on the susceptibility of wheat to *P. glumarum* and *Erysiphe graminis* DC., and of barley to *E. graminis*. He concluded that heavy applications of nitrogenous fertilizers increased the susceptibility of wheat to stripe rust, and of wheat and barley to powdery mildew. Potassium salts increased the resistance but did not counteract the effect of large amounts of nitrogen. Spinks concludes, however, that a highly resistant variety tends to remain resistant even when heavily fertilized with nitrogenous manures. Voelcker's results (37) were essentially similar to those of Spinks.

Hiltner (12), in 1914, noted that excessive use of Chile saltpeter greatly increased the amount of rusts on fall-sown wheat, but excessive amounts of ammonium sulphate did not increase them as much.

In 1915, Stranak (33), in his investigation of the effect of fertilizers on infection of wheat by *P. glumarum*, observed the greatest injury when the soil fertilization was unbalanced. Wheat grown on insufficiently fertilized soil suffered the greatest damage. But wheat overfertilized with nitrogen, especially when there was an insufficient supply of potash or phosphates, also was nearly always severely rusted.

Molz and Müller (21) in 1914 and 1916 concluded that potassium and phosphate fertilizers increased the resistance of wheat to stripe rust, and that the effect of nitrogen was uncertain.

Stakman (31) in 1913 made experiments on the effect of nutrient salts on the development of *P. graminis* on wheat plants in soil and sand cultures and found that excessive amounts of such fertilizers as nitrogen and phosphorus salts had no direct effect on the immunity or susceptibility of wheats. He considered temperature conditions and atmospheric humidity probably more important than soil conditions.

Armstrong (2), in 1922, pointed out that wheat fertilized with sodium nitrate was delayed and opportunity for the development of *P. glumarum* was increased. He attributed the effect of nitrogen to a lengthening of the growing period rather than to an actual increase of susceptibility.

Gassner (10), investigating the effect of fertilizers on the development of rusts of wheat, oats, barley, rye, and maize, in Uruguay, found that an apparent effect of fertilizers on the development of *P. graminis* was due to the effect on maturity of the host plants rather than to a change in the actual resistance.

Vavilov (36, p. 60-61) concluded that the apparent increased susceptibility of wheat to *Puccinia triticina*, when grown in soil fertilized with nitrogen, was due to increased development of leaf surface rather than to any change in real resistance. He grew 30 pure-line selections of spring wheat, of varying degrees of resistance to *P. triticina*, in unfertilized soil and also in soil heavily fertilized with potassium nitrate. There were only slight differences in the amount of rust on the plants in different plots, and resistant varieties remained resistant under all conditions.

Raines (23) studied the factors governing the virulence of *P. coronata* Cda., *P. secalina* Grove, *P. triticina* Erikss., and *P. sorghi* Schw. He was of the opinion that "a more catholic point of view in pathologic thought, recognizing that, for longer or shorter phases in the course of a disease, the relation between host and parasite may be highly mutualistic, would be of material value as a working concept in the study of diseases and in defining the practical problem of disease prevention and control."

This conclusion, in general, corroborates the evidence given by Ward (39), Arthur (3), Sheldon (27), Gibson (11), Stakman (31), Fromme (9), Mains (17), and others that the vigor of various rust parasites is likely to be directly proportional to the vegetative vigor of the host. The observations of Zavitz (41) on the effect of spacing also may be explained on the basis of greater vegetative vigor. Butler (6, p. 473-474) gives some evidence, however, that well nourished coffee bushes can withstand attacks by coffee rust, *Hemileia vastatrix* B. and Br., better than those which are growing under less favorable conditions.

It is apparent that the general consensus of opinion is that nitrogenous fertilizers increase the susceptibility of cereals to rusts, while potas-

sium and phosphorus salts tend to decrease it. Some investigators, notably Comes, apparently attribute the effect principally to changes in the chemical composition of the plants; others, such as Schindler (26, p. 168), Russell (25), and Miyake and Adachi (19) think the effect is rather on the mechanical composition of the cell walls. Schindler says that acid phosphates seem to cause the development of stronger tissues ("strammeres Gewebe"). Russell concludes that the walls of plants fertilized with excess nitrogen are thinner than they normally would be, and parasitic fungi therefore can penetrate more easily. Miyake and Adachi agree with Russell regarding the effect of nitrogen, and they report that potassium fertilizers strengthen the cell walls and thus increase resistance. Still others, especially Gassner (10), Freeman and Johnson (8), Stakman and Aamodt (32), and Armstrong (2), suggest that the effect of fertilizers is at least partly indirect.

Hurd's results (13) do not support the views of Comes that acidity of the host plant is the determining factor in resistance. She found no positive correlation between hydrogen-ion concentration and resistance. Neither could Hursh<sup>5</sup> find any correlation between sugar content of different varieties and the resistance to *Puccinia graminis*. On the other hand, there is some evidence that fertilizers may alter the structure of the host sufficiently to account partly, at least, for differences in the resistance of the same variety grown under different conditions.

That fertilizers may cause an apparent change in resistance by influencing rate of growth, density of stand, date of maturity, and yield can hardly be questioned.

#### OBJECTS OF THE INVESTIGATION

The objects of the investigation were: 1. To ascertain whether natural and artificial fertilizers alone and in various combinations, directly or indirectly, affect the degree of susceptibility of susceptible and resistant varieties of wheat to *Puccinia graminis* and *P. triticea*; 2. To ascertain what effect fertilizers have on the density of stand, the succulence of the plants, the degree of lodging, the date of maturity and similar characters which might indirectly influence the development of the rust; 3. To determine whether the same amount of rust will produce different degrees of injury when the plants are grown in different types of soil; 4. To determine whether plants receiving excessive nitrogen fertilization are especially liable to rust injury.

Attempts were made to predispose plants by means of barnyard manure, by growing them on clover and alfalfa soils and by adding nitrates directly to the soil. It was desired also to find out whether phosphates and potash could counteract the effect of large amounts of nitrogen and whether a combination of fertilizers could be found which would enable the plants to yield well in spite of rust. While the primary object was to determine, for practical purposes, the effect of normal amounts of fertilizers on the severity of rust attacks, attempts also were made to ascertain the effect on rust resistance of excessive amounts of fertilizers. As the work progressed, it became increasingly evident that it would be necessary to secure data on the effect of fertilizers on density of stand, succulence, degree of lodging, date of maturity and similar characters which indirectly might affect the development of rust without necessarily changing the actual resistance of the plants.

<sup>5</sup> Unpublished results.

After data had been obtained for several years from the field experiments, it became evident that the same amount of stem rust might not equally injure plants grown in different kinds of soil. It was suggested that plants very heavily fertilized with nitrogen might be especially liable to rust injury on account of succulence, weak straw, and high water requirement. Special investigations therefore were made on these phases of the problem.<sup>6</sup>

#### EXPERIMENTAL METHODS

Field experiments and observations were made for eight years on several types of soil and in several localities in Minnesota. Some pot experiments also were made. Four of the years were "rust years" and four were not.

The most extensive experiments were made on University Farm, and the Quinn farm, St. Paul. One series was made at Anoka; and observations were made on the regular fertilizer plats of the Division of Soils at Morris and at Crookston.

The soil on University Farm is the Hempstead silt loam. It varies considerably, but, in general, is well supplied with all necessary plant nutrients. It is described by Smith and Kirk (28) as follows: "The surface soil of the Hempstead silt loam consists of about 10 to 18 inches of black to dark brown silt loam, underlain by a subsoil consisting of brown to yellowish-brown-silty clay to silty clay loam, which extends to a depth of about 3 feet. Local variations include a somewhat open and loamy texture on the one hand, and a rather heavy and compact structure on the other. The substratum consists of a bed of rather clean gravel and sand." The series at University Farm was on the heavier and compact type, while that on the Quinn farm was on the more open and loamy texture.

The soil at Anoka is a loamy sand, often deficient in nitrogen. The following description is given by Smith et al. (29): "The Merrimac loamy sand consists of a brown to very dark brown loamy sand, 10 to 18 inches deep, underlain by a brown loamy sand subsoil and substratum."

The plats at Crookston were on loam which is very rich in organic matter and does not require artificial fertilizers. According to Mangum et al. (18), "The soil, to an average depth of 12 to 15 inches, consists of a dark brown to black silty clay which contains a very large percentage of organic matter. This material becomes slightly heavier as the depth increases and grades at about 12 inches into a heavy drab to gray silty clay, with a very finely stratified structure."

The fertilizer plats were laid out on uniform, level soil in order to avoid errors due to lack of uniformity in chemical composition, and to the washing of fertilizers from one plat to another. During the first four years, the size of the plats was one square rod. Three-foot alleys separated them from each other. In 1918, 1920, 1921, and 1922 observations were made on tenth-acre permanent fertilizer plats. Data on percentage of stem rust only were obtained from these plats.

The general plan of the commercial fertilizer plats in 1915, 1916, and 1917 on University Farm is shown in Table I. Modifications of this plan are indicated in the proper places.

<sup>6</sup> The results are published in the *Journal of Agricultural Research* in separate papers by Dr. Freeman Weiss and Dr. C. R. Hursh.



TABLE I.—General plan of commercial fertilizer plats in 1915, 1916, and 1917 on University Farm, St. Paul, Minn.<sup>a</sup>

Fertilizer.		Fertilizer, kind and amount in pounds per acre.						
Kind.	Amount per acre (pounds).	Sodium nitrate.			None.	Potassium chlorid.		
		1,000	500	250		1,000	500	250
Acid phosphate. ....	2,000	1	2	3	4	5	6	7
	1,000	8	9	10	11	12	13	14
	500	15	16	17	18	19	20	21
	None.	22	23	24	25	26	27	28

<sup>a</sup> The plats are numbered from left to right in all plat plans.

It will be noted from Table I that there were 28 plats in the regular series. The fertilizers, and amounts applied, are indicated at the left of the horizontal columns and at the tops of the vertical ones. For instance, plat 3 (reading from the upper left-hand plat to the right), received an application of acid phosphate at the rate of 2,000 pounds per acre and sodium nitrate at the rate of 250 pounds per acre. Plat 4 received only phosphate, and plat 25 is a control. Each of the three fertilizers was used alone in three concentrations, and combinations were made between acid phosphate and each of the other fertilizers. This plan was changed slightly in some of the experiments in order to make other combinations of fertilizers, and, on the poorer soils, a complete fertilizer also was used. In addition, experiments were made with barnyard manure in combination with other fertilizers. In 1915, plats were established at University Farm on cabbage, clover, and alfalfa soils. The details will be given under each experiment, as they vary considerably.

The principal commercial fertilizers used in the tests made during the first four years were acid phosphate, sodium nitrate and potassium chlorid. In 1916, potassium chlorid could not be obtained, so potassium sulphate was used instead. The barnyard manure used was well rotted.

The acid phosphate was worked thoroughly into the soil before seeding, while potassium chlorid and potassium sulphate were applied as a top dressing immediately after seeding. The sodium nitrate was applied as a top dressing shortly after the plants had emerged from the soil, and applications were made at two-week intervals, at the rate of 250 pounds per acre, until the total amount had been applied to each plat. The manure, which always refers to well-rotted barnyard manure, was applied before seeding and was thoroughly worked into the soil.

A resistant and a susceptible variety of wheat were sown in each plat in most of the experiments at University Farm. The varieties used and their average degree of resistance to stem rust under field conditions will be given under the description of each experiment.

Under ordinary conditions a severe epidemic of stem rust is not sure to occur every year. Owing to location, no attempt was made to induce the development of artificial epidemics on the wheat in the plats on the Quinn farm, or at Anoka and the Crookston substation. Definite measures, however, were taken to produce a severe epidemic on the



plats at University Farm. By the method described below a severe epidemic was obtained each year.

Common barberry bushes, covered with wheat straw containing teliospores, were grown in the field. This straw was placed around the barberry bushes late in the fall and was allowed to remain until the leaves had unfolded in the spring. Abundant aecia developed on the barberry leaves. Winter wheat was sown early in the fall around the barberry bushes. This resulted in the production of urediniospores on the winter wheat early in the spring. The wheat plants in the fertilizer plats were then sprayed with a water suspension of aeciospores from the barberry leaves and urediniospores from the winter wheat. The plants were sprayed every other day after sunset, or during the day in wet, cloudy weather, until a heavy epidemic was assured. In addition, flower pots, containing wheat seedlings, well covered with uredinia, were placed in the plats. Large quantities of rusted material were developed in the greenhouse and there held in readiness to be placed in

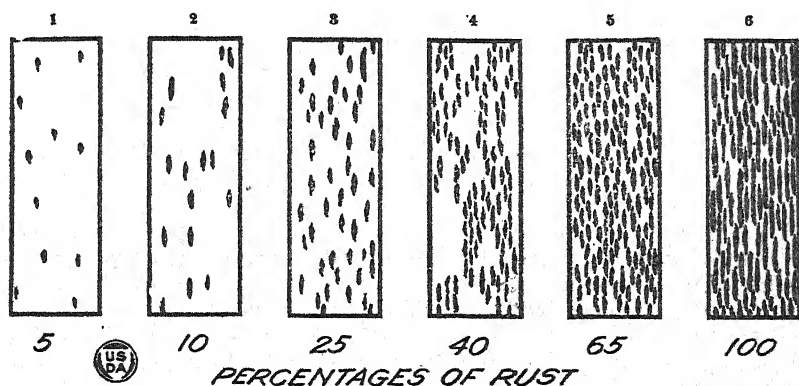


FIG. 1.—Scale for estimating percentage of stem rust. The shaded spots represent rust, and the figures represent approximately the rust percentages computed on the basis of the maximum amount of surface covered by rust as shown in No. 6, which represents 37 per cent of surface covered with rust pustules and is arbitrarily selected as 100 per cent. Other percentages are based on No. 6. (From Office of Cereal Investigations, United States Department of Agriculture.)

the field at the proper time. Spore suspensions for spraying also were made from this material. Hand inoculations were made in the field in the same manner as in the greenhouse. For this purpose control rows of a susceptible variety were sown along the side of the plat. After the inoculum had been placed on the seedlings, they were covered with a bell jar, or inverted flower pot, for 48 hours, the soil being kept wet around the jar or pot during this period.

The scale (fig. 1) used in the stem-rust investigations made by the Office of Cereal Investigations of the United States Department of Agriculture, was used in estimating the percentage of rust.

The final percentages of rust infection were estimated just before the plants ripened. They were made by several experienced observers, some of whom did not know the disposition of fertilizers in the plats. The estimates made by the different individuals did not vary over 5 per cent for any one plat.

Estimates of the degree of lodging, the severity of stem rust on the peduncle, sheath and leaves, the amount of seed shriveling, and the

degree of crinkling are indicated in the tables of results by the following figures:

- 0 per cent = none.
- 20 per cent = very light.
- 40 per cent = light.
- 60 per cent = medium.
- 80 per cent = heavy.
- 100 per cent = very heavy.

### EXPERIMENTAL RESULTS

#### EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1914

The experiments in 1914 were preliminary. Nine plats of 1 square rod each, separated from each other by 3-foot alleys, were laid out on fairly light Hempstead clay loam on University Farm, St. Paul. Acid phosphate, at the rate of 480 pounds per acre, was added to three of these plats; sodium nitrate was added to three others at the rate of 360 pounds per acre; and three remained untreated as controls. Three varieties of wheat were sown, one on each of the square-rod plats to which the same fertilizer had been applied. These varieties were Glyndon Fife, C. I. 2873,<sup>7</sup> a hard red spring wheat, which is susceptible to stem rust in the field in Minnesota; Iumillo, C. I. 1736, a very resistant durum, and a moderately resistant selection from a durum-common cross. The seed was sown late and a heavy epidemic of stem rust developed.

As will be seen from Table II, the amount of stem rust which developed on the same variety in the different plats varied very slightly. On Glyndon Fife, the range was from 80 to 85 per cent; on Iumillo, it was from 15 to 20 per cent, and on the hybrid from 35 to 50 per cent. The lowest percentage of stem rust on Glyndon Fife was in the plat fertilized with acid phosphate, but on Iumillo and the hybrid the highest percentage was in the phosphate plats.

While the results are only indicative, it is quite clear that none of the fertilizers had an appreciable effect on the amount of stem rust. There was so little leaf rust that no data were obtained on its relative prevalence.

TABLE II.—*The percentages of stem rust on Glyndon Fife, Iumillo, and durum-common hybrid wheat in 1914, in the commercial fertilizer plats on University Farm, St. Paul, Minn.*

Kind and amount of fertilizer in pounds per acre.	Variety of wheat and percentage of stem rust.		
	Glyndon Fife.	Iumillo.	Semiresistant hybrid.
	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Control, none.....	85	15+	35
Acid phosphate 480.....	80+	20—	50
Sodium nitrate 160.....	85	20—	35

<sup>7</sup> Accession number of the Office of Cereal Investigations, U. S. Department of Agriculture.

## EXPERIMENTS IN THE GREENHOUSE IN 1914-15

As there was no observable effect of the different fertilizers on the amount of stem rust in the field during the summer of 1914, experiments were made in the greenhouse during the winter to determine whether an effect could be brought about under controlled conditions.

Wheat, oats, barley, and rye were sown in ordinary greenhouse soil, made up of sand, leaf mold, and black loam in 4-inch pots, where the plants were grown to maturity. Fertilizers were added to some and others were left as controls.

Wheat plants were inoculated with *Puccinia graminis tritici* and became quite uniformly infected with rust. The lightest infection was on plants which had been fertilized with acid phosphate. It was evident, however, that too much fertilizer had been used, since the plants were subnormal in vigor and size. Even when so much acid phosphate was used, however, that the plants were injured, the development of stem rust was not greatly inhibited.

Several pots of barley were fertilized with sodium nitrate and others with potassium chlorid. About 20 plants were inoculated in each series. Practically 100 per cent became infected. The uredinia, however, on the plants which had been fertilized with sodium nitrate were larger than were those on plants which had been fertilized with potassium chlorid. As there seemed to be some effect of fertilizers on the development of rust on plants growing in the greenhouse, an attempt was made to ascertain whether fertilizers would influence the development of stem rust on a semicongenial host.

Rye was inoculated with *Puccinia graminis tritici*, to which it is highly resistant. Some of the plants were grown in soil that had been heavily fertilized with sodium nitrate and others were grown in unfertilized soil. Two of the 13 plants which had been fertilized with nitrogen became infected, and one of the 19 grown in ordinary soil also developed uredinia. There were very few uredinia on any of the plants, but the individual uredinia were slightly larger on the plants which had been fertilized with sodium nitrate.

In order to get further data on the effects of fertilizers on the development of rust on a very highly resistant host, 58 barley plants growing in soil which had been heavily fertilized with sodium nitrate, and 40 growing in soil which had been heavily fertilized with potassium chlorid, were inoculated with *Puccinia graminis avenae*. Barley is almost immune from this form of stem rust although minute uredinia, usually smaller than a pinhead, sometimes are formed. One plant in the nitrate series became infected, and two of those in the potassium series developed uredinia. All of the uredinia were extremely small. The resistance of the barley plants to *Puccinia graminis avenae* certainly was not broken down by excessive fertilization with nitrogen, nor did the resistance seem to be increased by heavy fertilization with potassium chlorid.

In order to get still more information regarding the effect of stem rust on a congenial host, experiments were made with *Puccinia graminis avenae* on oats. Acid phosphate was added to the soil in some of the pots, while no fertilizer was added to others. So much acid phosphate was added that the tips of the leaves burned slightly. The degree of infection was about equal in both series. However, sometime after the plants had become infected, the uredinia seemed to be larger on the plants

which had not been fertilized with acid phosphate. This seemed to be quite natural, as the fertilized plants were small and unthrifty. They had been injured by excessive fertilization, but, even then, stem rust developed fairly well.

#### FIELD EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1915

The field plats in 1915 were laid out on University Farm on rather heavy Hempstead loam soil<sup>\*</sup> which had grown alfalfa for the preceding 15 years. Each plat was 1 square rod in extent and all were separated from each other by 3-foot alleys. The general arrangement is shown in Table III. The fertilizers were applied as indicated under "Experimental methods."

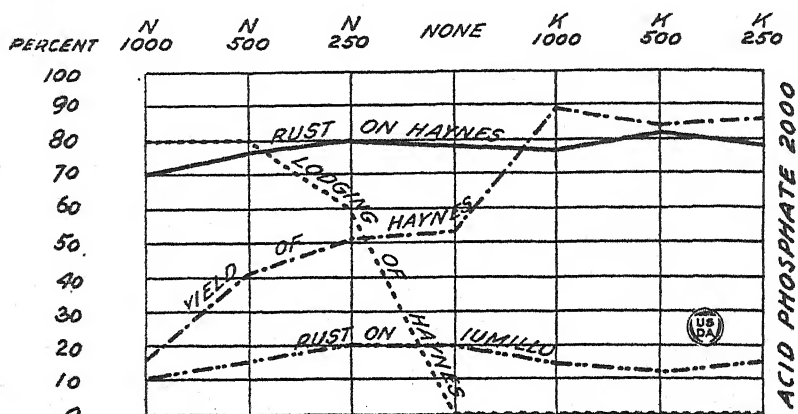


FIG. 2.—Graph showing the percentages of stem rust on Haynes Bluestem and on Iumillo and the percentage of lodging and acre yield in bushels (31 bushels=100 per cent) of Haynes Bluestem grown in plats which had received acid phosphate at the rate of 2,000 pounds per acre plus different amounts, in pounds, of sodium nitrate (N) and potassium chlorid (K) in the commercial fertilizer experiments in 1915 on University Farm, St. Paul, Minn.

- rust on Haynes Bluestem.
- - - lodging of Haynes Bluestem.
- ..... yield of Haynes Bluestem.
- · - · - rust on Iumillo.

On May 6, Haynes Bluestem, C. I. 2874, was sown on half of each plat; and Iumillo, C. I. 1736, was sown on the other half. Haynes Bluestem is a hard red spring wheat, one of the best milling wheats grown in that region. But it is extremely susceptible to black stem rust, as is indicated by the fact that this variety has had an average infection of 70 per cent in the Minnesota rust nursery during the past eight years. Iumillo is a highly resistant durum on which the average percentage of rust in the Minnesota rust nursery from 1910 to 1920 was 10 per cent.

The seed was sown on May 6. Differences in the character of growth of the wheat in the different plats became apparent soon after the plants came up. The heaviest stand was on the nitrate plats, and the density of stand was in direct proportion to the amount of nitrogen applied. The plants were very succulent and soon began to lodge rather badly. The plants in the nitrogen plats also were a much darker green than those in the other plats. There was very little difference in the general

<sup>\*</sup> See description of this soil under "Methods."

appearance of the plants in any of the plats except those which had been fertilized with nitrogen.

On July 1, stem rust began to appear on Haynes Bluestem. None had yet appeared on Iumillo. By July 27, Haynes Bluestem was uniformly infected with stem rust, but there were no apparent differences in the degree of infection in the different plats. On August 25, the plants were practically ripe and final observations were made. The results are given in Table III, which also is a diagram of the plats and their treatment. Figure 2 presents graphically the results from the series of seven plats receiving 2,000 pounds of acid phosphate and various other treatments.

TABLE III.—The effect of commercial fertilizers on the percentages of stem rust on Iumillo and Haynes Bluestem wheats; and on the degree of lodging and yield of Haynes Bluestem in 1915 on University Farm, St. Paul, Minn.

Infection, lodging, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium chlorid.		
			1,000	500	250		1,000	500	250
Per cent stem rust, Haynes Bluestem.....	Acid phosphate.	2,000	70	76	80	78+	77	82	78
Per cent stem rust, Iumillo.....			10	15	20	20—	15	12—	15
Per cent lodging, Haynes Bluestem.			80	80	60	0	0	0	0
Bushels per acre, Haynes Bluestem.			5	12.8	15.8	16.6	27.9	26.2	26.9
Per cent stem rust, Haynes Bluestem.....		1,000	76	78+	80	80+	85	86	85—
Per cent stem rust, Iumillo.....			20—	15	18	20+	20	20	20
Per cent lodging, Haynes Bluestem.			60	60	60	40	60	60	40
Bushels per acre, Haynes Bluestem.			8.2	13.9	14.1	17.8	22.7	22.9	26.1
Per cent stem rust, Haynes Bluestem.....		500	83—	85+	82	83+	87	85—	88
Per cent stem rust, Iumillo.....			23	18	20	25—	15	15—	20—
Per cent lodging, Haynes Bluestem.			40	40	40	20	0	20	0
Bushels per acre, Haynes Bluestem.			6.5	14.3	17.1	19.1	19.3	25.5	31
Per cent stem rust, Haynes Bluestem.....		None.	80	82—	85+	83	81+	80+	86+
Per cent stem rust, Iumillo.....			22	22—	25—	25	18	15—	20
Per cent lodging, Haynes Bluestem.			40	40	0	0	0	0	0
Bushels per acre, Haynes Bluestem.			7.8	16.2	19.8	22.8	20.7	21.4	20.5

It is quite evident from Table III that there is no definite correlation between the kind and amount of fertilizers applied and the amount of stem rust on the plants. The extreme range in percentage of stem rust is from 70 to 88 per cent. It so happens that the lowest percentage was in the plat which had received 1,000 pounds of sodium nitrate and 2,000 pounds of acid phosphate, while the highest percentage was in the plat which had received 500 pounds of acid phosphate and 250 pounds of potassium chlorid.<sup>9</sup> The heaviest infection, then, was in one of the

<sup>9</sup> The figures given always indicate the rate per acre at which the fertilizers were applied; for convenience the rate is given rather than the actual amount applied to each square-rod plat.

plots in which the plants theoretically should have been protected by the action of fertilizers. The percentage of infection in the plot which had received 1,000 pounds of sodium nitrate alone was 80, while in the one which had received 250 pounds it was 85. Certainly there seems to be no evidence that very heavy fertilization with nitrogen predisposed plants to stem rust, nor that acid phosphate and potassium chlorid protected them from it.

There are very striking differences, however, between the degree of infection on Haynes Bluestem and that on Lumillo. The average percentage of infection on Bluestem was approximately 80, while that on Lumillo was about 20. It is quite evident that the resistance of Lumillo was not broken down by excessive fertilization with sodium nitrate. In fact, there was more rust on the plants in the plots which had received no fertilizer whatever than there was on those in the plots which had received 1,000 pounds of sodium nitrate.

The results are particularly striking, as the soil on which the experiments were made was well supplied with nitrogen. It will be noted from Table III that the addition of nitrogen to the soil not only did not result in increased yields but actually caused a decrease, indicating quite clearly that the soil was not deficient in nitrogen. Any nitrogen which was added, therefore, was excessive.

While there was no correlation between kind and amount of fertilizers applied and the amount of stem rust which developed, there was a definite correlation between fertilization and lodging and yield. (See fig. 2.) The degree of lodging was much greater in the nitrogen plots than in any of the others and the yields were lower. The lowest yield, 5 bushels per acre, was in plot 1, which had received 2,000 pounds of acid phosphate and 1,000 pounds of sodium nitrate, and in which the degree of rust infection was lower than that in any of the other plots. The highest yield, 31 bushels per acre, by a rather remarkable coincidence, was in the plot in which the rust infection was 88 per cent, or higher than that in any of the other plots. It is a rather striking fact that the yield was always inversely proportional to the amount of nitrogen applied.

It is well known that wheat which has been overfertilized with nitrogen is likely to yield poorly. Not only does it often lodge badly but it frequently dries out, a phenomenon spoken of by farmers as "burning out." This burning out was quite conspicuous in the plots fertilized with nitrogen. It was supposed that there might be more rust on the plants which lodged badly in the nitrogen plots than on those which remained erect, but apparently such was not the case.

It is worthy of attention that the amount of rust on the plants is not necessarily an index of the damage which may be done. It seems quite likely that much of the injury usually attributed to rust on plants heavily fertilized with nitrogen probably is due to the direct effect of nitrogen rather than rust. For instance, wheat growing in soil which had received 1,000 pounds of sodium nitrate per acre had 80 per cent of rust infection and yielded only 7.8 bushels per acre, while that which had received 500 pounds of acid phosphate and 250 pounds of potassium chlorid had 88 per cent of rust but yielded 31 bushels per acre. It will be seen by referring to figure 1 (p. 347) that 88 per cent of infection is extremely heavy. The fact that plants so heavily infected with rust can produce well simply indicates that the visible amount of rust on the plants is no accurate measure of the damage done. It seems clear that

plants which have been fertilized properly can sometimes yield well even when heavily infected with stem rust.

There appeared to be a direct correlation, however, between the kind and amount of fertilizer applied and the percentage of infection by orange leaf rust, *Puccinia triticina* (Table IV). The percentages are not high because Haynes Bluestem is moderately resistant and Iumillo is very resistant in the field. The range in percentage of infection on Haynes Bluestem was from 10 to 40, the highest being in the nitrogen plats. The average percentage of infection in those plats which had received only nitrogen was 36; in those which had received nitrogen and phosphorus it was 29; in the phosphate plats, 22; in the potash plats, 17; in the phosphorus-plus-potassium plats, 21; while in the unfertilized control plat, it was 23. There was only a trace of leaf rust on Iumillo in all plats except the control and those which had received sodium nitrate. The highest percentage of infection was 10 in the plat receiving 1,000 pounds of nitrogen.

Evidently fertilization with sodium nitrate caused increased development of leaf rust, and phosphorus and potassium had some tendency to prevent its development, but it is quite likely that the effect may have been indirect. On the natural fertilizer plats there appeared to be no correlation between fertilization and development of leaf rust.

Unfortunately, very little leaf rust developed in any of the years subsequent to 1915, so additional data were not obtained and definite conclusions could not be drawn.

TABLE IV.—The effect of commercial fertilizers on the degree of infection of *Puccinia triticina* in 1915 on Haynes Bluestem and Iumillo wheats in the plats on University Farm, St. Paul, Minn.

Infection.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.								
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium chlorid.				
			1,000	500	250		1,000	500	250		
		Acid phosphate.	Pounds.								
Percentage of leaf rust, Haynes Bluestem. ....			2, 000	{	25	27	22	15	10	15	15
Percentage of leaf rust, Iumillo. ....					5	2	1	1	1—	1—	1—
Percentage of leaf rust, Haynes Bluestem. ....			1, 000	{	30	30	25	25	15	20	20
Percentage of leaf rust, Iumillo. ....					5	3	1—	1—	1	1—	1—
Percentage of leaf rust, Haynes Bluestem. ....			500	{	40	35	30	25	12	15	15
Percentage of leaf rust, Iumillo. ....					5	3	1	1	1—	1—	1—
Percentage of leaf rust, Haynes Bluestem. ....			None.	{	40	40	27	23	20	15	15
Percentage of leaf rust, Iumillo. ....					10+	5	7	3	1	1—	1—

#### EXPERIMENTS WITH COMMERCIAL FERTILIZERS PLUS NATURAL FERTILIZERS IN 1915

As previously outlined, the second method used in these experiments consisted in growing wheat on soil enriched with such natural fertilizers as barnyard manure, clover, and alfalfa, both alone and in various com-

binations with commercial fertilizers. The commercial fertilizers used were sodium nitrate, acid phosphate, and potassium chlorid. The soil was of the same type as that on which the experiments with commercial fertilizers alone were made. The plats which previously had grown cabbage and clover were contiguous to each other, while those on the alfalfa soil were a few rods distant.

The methods used in laying out the plats and applying the fertilizers were similar to those employed in the experiments with commercial fertilizers. The plats were 1 square rod in area and separated from each other by 3-foot alleys.

The commercial fertilizers were applied as indicated under the discussion of general methods.

Three series of plats were used. The soil in one series had grown cabbage the previous year, that in another had been in clover for several years, and that in the third had been in alfalfa for a like period. The ground was plowed, disked, and harrowed thoroughly before the seed was sown. Well-rotted barnyard manure, at the rate of 50 tons per acre, was applied to one plat in each series; 50 tons of manure, plus 500 pounds of sodium nitrate, per acre to one plat; 50 tons of manure, plus 1,000 pounds of acid phosphate, per acre to one plat; and 50 tons of manure, plus 1,000 pounds of potassium chlorid, per acre to one plat. The control plats received neither manure nor commercial fertilizers.

One-half of each plat was sown to Haynes Bluestem wheat, the other half to a hybrid (No. 4 × 942.10) originated in the Minnesota rust nursery. This hybrid was a cross between a durum (Kubanka, C. I. 2094) and Haynes Bluestem. It was a fixed type and averaged 30 per cent of rust in the Minnesota rust nursery in the five years from 1912 to 1917.

The disposition of the plats and the kind of soil and fertilizers used are shown in Table V. This table also gives the percentages of stem-rust infection for both varieties of wheat and the total number of days between the time Haynes Bluestem was sown and the time it ripened.

The stem-rust epidemic was assured by spraying with a suspension of spores such as had been used in the commercial fertilizer plats.

On July 1 the stand in the control plats on the alfalfa and clover soils was heavier than that in the control plat on the cabbage soil. The plants in all of the manured plats also were quite succulent and dark green in color. The commercial fertilizers apparently had influenced the character of growth but little up to this time.



TABLE V.—The effect of commercial and natural fertilizers on the percentages of stem rust and leaf rust, and the total number of days for maturity for Haynes Bluestem and the resistant hybrid in 1915 on University Farm, St. Paul, Minn.

Infection and growing period.	Fertilizer, kind and amount per acre.				
	50 tons of manure per acre, plus—				None
	Nothing.	Sodium nitrate, 500 pounds.	Acid phosphate, 1,000 pounds.	Potassium chlorid, 1,000 pounds.	
CABBAGE SOIL					
Percentage of stem rust, Haynes Bluestem....	87	88	85	90	70
Percentage of stem rust, hybrid.....	30	30	37	40	40
Percentage of leaf rust, Haynes Bluestem....	7	15	10	12	15
Percentage of leaf rust, hybrid.....	27	27	25	30	20
Days for maturity of Haynes Bluestem.....	115	114	114	114	94
CLOVER SOIL					
Percentage of stem rust, Haynes Bluestem....	85	80	86	87	70
Percentage of stem rust, hybrid.....	40	43	48	46	35
Percentage of leaf rust, Haynes Bluestem....	12	15	12	12	20
Percentage of leaf rust, hybrid.....	30	27	25	20	28
Days for maturity of Haynes Bluestem.....	113	114	114	113	104
ALFALFA SOIL					
Percentage of stem rust, Haynes Bluestem....	80	74	76	78	70
Percentage of stem rust, hybrid.....	38	38	35	36	25
Percentage of leaf rust, Haynes Bluestem....	25	30	25	25	35
Percentage of leaf rust, hybrid.....	37	35	32	35	35
Days for maturity of Haynes Bluestem.....	114	115	115	115	110

On July 1 a few uredinia of stem rust were found on Haynes Bluestem but not on the hybrid. On July 27 Bluestem in all plats was quite uniformly infected with stem rust, although the percentage of infection on the plants in the cabbage soil was slightly higher than that on those in the clover soil, and those in the clover soil were slightly more severely affected than those on the alfalfa soil. The cause of these differences lay in the fact that the plats on the cabbage soil were nearest to heavily rusted barberry bushes. Therefore, a comparison between the amount of stem rust on plants in cabbage soil and those in alfalfa and clover soils can only be general. The degree of rust infection in these different series is correlated with the proximity of the plats to the rusted barberry bushes. On August 6 stem rust was spreading rapidly to stems and leaves on Bluestem in all the plats, but the spread on the hybrid was only moderate. The hybrid lodged severely in all of the manured plats, while the Bluestem lodged moderately in a few of them. Both varieties stood up perfectly in all of the control plats, except one in which the hybrid lodged somewhat.

From the data presented in Table V it at first appears that manure increased the susceptibility of the plants. But it is probable that this apparent increase of susceptibility was due partly at least to later maturity of the wheat, giving the rust a longer time to spread. The plants in the manured plats on the cabbage soil ripened 20 days after those in the control plats; those on the clover soil 10 days after those in the control plat; and those on the alfalfa soil 5 days after those in the control plat. There appears to be a complete correlation between the increase in percentage of stem rust on the Haynes Bluestem plants in the manured plats over that on those in the control plats, and the increased number of days required for maturity of the plants in the manured plats over those in the control plats. On the other hand, the percentage of infection in all of the control plats was 70, in spite of the fact that the plants in the different plats matured on different dates. The Bluestem in the control plat of the cabbage-soil series was mature on August 15,

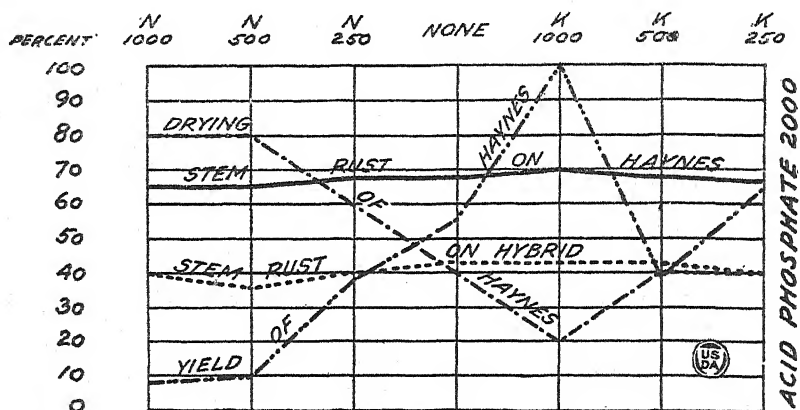


FIG. 3.—Graph showing the percentage of stem rust on Haynes Bluestem and on the hybrid and percentage of drying and acre yield in bushels (7.2 bushels=100 per cent) of Haynes Bluestem grown in plats which had received acid phosphate at the rate of 2,000 pounds per acre plus varying amounts in pounds of sodium nitrate (N) and potassium sulphate (K) in the commercial fertilizer experiments in 1916 on University Farm, St. Paul, Minn.

- stem rust on Haynes Bluestem.
- - - stem rust on hybrid.
- ..... drying of Haynes Bluestem.
- · - · - yield of Haynes Bluestem.

in the control plat of the clover-soil series on August 22, and in the control plat of the alfalfa-soil series on August 28. Up to August 28, therefore, the dates of maturity seemed to have no effect on the amount of rust. After this date, however, rust again spread rather rapidly on those plants which remained green, but it is impossible to say just how much effect delayed maturity had on the percentage of rust in this series.

There is no definite correlation between fertilization and the percentage of infection of *P. triticea*, which is in sharp contrast with the results obtained on the commercial fertilizer plats. The hybrid appeared more susceptible than Haynes Bluestem, but the highest percentage of infection, 35 for both varieties, was on unfertilized alfalfa soil.

#### EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1916

The commercial fertilizer plats in 1916 were the same plats actually used in 1915. A resistant hybrid wheat was used instead of Iumillo, however, and potassium sulphate was used instead of potassium chlorid.

The size and position of the plats and the rate of application of fertilizers were exactly the same as in 1915. (See Table VI.)

The hybrid (No. 5 × 2223), used in place of Lumillo, was a cross between a durum, Kubanka, C. I. No. 2094, and a common hard red spring variety, Preston, C. I. 3328. It was a fixed type, in the  $F_0$  generation, originated by Freeman and Johnson. The average amount of stem rust on the hybrid in the Minnesota rust nursery from 1912 to 1916 was 30 per cent. It is slightly more susceptible, therefore, than Lumillo.

Both varieties were sown on May 13, which is rather late for the district. However, the spring was late. The temperatures were abnormally low during April, May, and June, while the precipitation was abnormally high. During July and August, however, the conditions were reversed. These abnormal climatic conditions undoubtedly affected the development of stem rust, the date of maturity of the plants, and the yield. The results obtained, as well as the plan of the experiment, are shown in Table VI. Figure 3 shows the results in the seven plats of the series receiving 2,000 pounds of acid phosphate.

TABLE VI.—The disposition of the commercial fertilizer plats and the percentages of stem-rust infection on Haynes Bluestem wheat and the hybrid, and the degree of drying and yield in bushels per acre of Haynes Bluestem in 1916 on commercial fertilizer plats on University Farm, St. Paul, Minn.

Infection, drying, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.				Potassium sulphate.		
			None.			None.	None.		
			1,000	500	250		1,000	500	250
Percentage of stem rust, Haynes Bluestem.....	Acid phosphate.	2,000	65.0	65.0	68.0	68.0	70.0	68.0	67.0
Percentage of stem rust, hybrid....			40	36	40	43	43	43	40
Percentage of drying, Haynes Bluestem.....			80	80	60	40	20	40	40
Bushels per acre, Haynes Bluestem..			0.6	0.7	2.7	4	7.2	2.8	4.7
Bushels per acre, hybrid.....			6.6	6.9	7.8	14.3	18.8	18.6	16.8
Percentage of stem rust, Haynes Bluestem.....		1,000	65	66+	66+	66	70	70	70
Percentage of stem rust, hybrid....			40	38	40	42	43+	43	43
Percentage of drying, Haynes Bluestem.....			80	80	60	40	40	40	40
Bushels per acre, Haynes Bluestem..			0.6	0.5	1.0	1.3	4.6	1.7	3.5
Bushels per acre, hybrid.....			5.2	6.9	7.5	13.7	18.6	17.4	17.9
Percentage of stem rust, Haynes Bluestem.....		500	66	66+	67	69	70	70+	70+
Percentage of stem rust, hybrid....			45	41+	42	43+	45	45	45
Percentage of drying, Haynes Bluestem.....			60	80	80	40	40	40	40
Bushels per acre, Haynes Bluestem..			0.3	0.5	0.7	1.4	2.7	1.7	2.8
Bushels per acre, hybrid.....			7.4	9.6	9.1	14.6	20.3	13.0	19.4
Percentage of stem rust, Haynes Bluestem.....		None.	67+	65	67+	70	71	70	70+
Percentage of stem rust, hybrid....			43	42	42	43+	45	43+	44
Percentage of drying, Haynes Bluestem.....			80	60	60	40	40	40	40
Bushels per acre, Haynes Bluestem..			0.5	0.5	0.7	1.8	2.2	1.4	2.8
Bushels per acre, hybrid.....			7.7	10.0	8.1	16.1	16.8	11.2	16.7

Stem rust first began to appear on June 16, after which it developed and spread rapidly and uniformly until checked by the hot, dry weather. The growth of the wheat plants also was checked by the drought, the plants in the plats treated with nitrogen plus acid phosphate being injured most severely. As there was some drying and premature ripening, the rust did not become as abundant as it had in 1915. It will be noted that in 1916 the period between sowing and harvesting was only 85 days, while in 1915 it was 116 days. The range in percentage of stem-rust infection on Haynes Bluestem in 1916 was from 65 to 70 per cent and on the resistant hybrid it was from 36 to 45 per cent. The narrow range may have been due partly to the fact that the plants ripened prematurely. It is interesting to note that, as in 1915, the highest percentage of stem rust on Haynes Bluestem was not in the nitrate plats. In fact, the average percentage of infection was lower in those plats which had been fertilized with nitrogen alone than in the control plat and in those which had received acid phosphate and potassium sulphate. The average percentage of stem rust also was slightly higher on the hybrid in the phosphate and potassium than in the nitrogen plats. It is perfectly clear, therefore, that excessive fertilization with nitrogen failed entirely to predispose either the susceptible Haynes Bluestem or the resistant hybrid to stem rust; nor did phosphorus and potassium protect them. Whatever slight effect there may have been appeared to be just the opposite of what one would expect. There was a very definite lack of correlation between fertilization and the development of stem rust (see Table VI and fig. 3), a fact for which weather conditions probably were partly responsible.

About a week after the plants had headed, the weather became very hot and dry. The growth of the plants, especially in the nitrate and phosphate plats, was severely checked and many of them began to dry up and crinkle. The crinkling was fairly uniform and could not be attributed entirely to the effect of fertilizers, although it was consistently worse in the nitrate plats than in any others. As would be expected, "burning out," due to heavy fertilization with nitrogen, is aggravated by hot dry weather. As "burning out" really means premature ripening, nitrogen actually may cause a decrease in the amount of rust in years when the weather is hot and dry. But in years when the growing conditions are favorable it may increase rust infection by prolonging the growing period. The degree of shriveling of seed was in direct proportion to the amount of drying, and the yield, in general, was inversely proportional to the amount of shriveling. For instance, the acre yield of Haynes Bluestem was less than 1 bushel in all of the plats in which the degree of drying was 80, while on all of those in which the drying was less than 60, the yield was over 1 bushel per acre.

The yields in 1916 were deplorably low, of course, as they were generally throughout the hard red spring wheat region. For instance, in 1915, the average acre yield in Minnesota was 17 bushels, in South Dakota 17, and in North Dakota 18.2 bushels. In 1916, they were 7.5, 6.3, and 5.5 bushels, respectively (35, p. 593). This was attributed principally to the terrific stem-rust epidemic, the most destructive since 1904. It is interesting, however, to note that the average percentage of stem rust on all plats in 1915 was considerably higher than it was in 1916. But the yields in 1915 were very much higher than they were in 1916. In 1915 the lowest yield in the commercial fertilizer plats was 5 bushels

per acre and the highest was 31 bushels. In 1916 the lowest yield was only 0.3 of a bushel per acre and the highest only 7.1 bushels. Either the drought alone caused much of the damage in 1916, or the drought aggravated the rust damage. As Weaver (40) has shown that the rate of transpiration of rusted cereal plants is considerably greater than that of rust-free plants, it seems probable either that the loss of water or the water requirement is an important factor in rust damage. This would explain why plants fertilized with nitrogen apparently or actually are injured more by rust than are other plants. The susceptibility of the plants to rust is not necessarily increased by the nitrogen, but the rust is especially dangerous to such plants, because it increases the water requirement which already is too high for safety. That the great damage to the Haynes Bluestem in the commercial fertilizer plats was caused by a combination of drought and stem rust, and not by drought alone, is suggested by the fact that the resistant hybrid yielded from two to about twelve times as much as the Bluestem. (See Table VI.) It is possible, however, that the hybrid may be somewhat drought resistant. While it is difficult to differentiate clearly between drought injury and rust injury, it is quite evident that the degree of rust infection is not necessarily an index of the amount of injury it will cause.

The results obtained in 1915 and 1916 indicate clearly that wheat may yield well in spite of heavy stem-rust infection, if weather conditions are favorable to the crop. On the contrary, a moderate rust attack either causes or appears to cause severe damage when weather and soil conditions are unfavorable to the wheat. It is probable also that the effect of fertilizers may be different under different climatic conditions. In a hot, dry year, wheat fertilized with nitrogen may "burn out," thus checking the spread of stem rust; while in a cool, moist season, maturity may be delayed by nitrogenous fertilizers, thus giving the rust more time in which to develop.

#### EXPERIMENTS WITH COMMERCIAL FERTILIZERS PLUS NATURAL FERTILIZER IN 1916

The general plan of the plats treated with commercial plus natural fertilizer is shown in Table VII. The plats were on heavy Hempstead clay loam on University Farm, which had grown alfalfa for several years previously. Haynes Bluestem and the same hybrid wheat which was used in 1915 also were used in this experiment. Each variety occupied 1 full square rod instead of half that area as in 1915. The general conditions of the experiment were the same as those for the commercial fertilizer experiments in 1916.

TABLE VII.—*The disposition of the plats treated with commercial plus natural fertilizer and the percentages of rust on Haynes Bluestem and the hybrid in the plats on University Farm, St. Paul, Minn., in 1916*

Infection.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.			
	Kind.	Amount per acre.	Sodium nitrate. 500.	Potassium sulphate 1,000.	None.	Dried blood 650.
Percentage of stem rust, Haynes Bluestem.	Manure.	10 tons...	58	60	61	63
Percentage of stem rust, hybrid . . . . .			40	41	43	42
Percentage of stem rust, Haynes Bluestem.		25 tons...	58	58	61	63
Percentage of stem rust, hybrid . . . . .			40	40	43	42
Percentage of stem rust, Haynes Bluestem.		Control...	55	.....	.....	.....
Percentage of stem rust, hybrid . . . . .			38	.....	.....	.....

The plants on the manured plats were uniform and were much more vigorous and darker green in color than those on the control plat. One week after heading out, the plants were injured by drought. The peduncles of the plants broke over or crinkled at the point where they emerged from the sheath. This occurred so uniformly on all the plats that it could not be attributed entirely to the effect of the fertilizers. The same effect was noted on the plats in the experiment with the commercial fertilizers for this same year.

The plants lodged badly on all the manured plats, while there was no lodging in the control plat. The average percentage of lodging in the plats receiving 10 tons of manure per acre was 40, while in those receiving 25 tons it was 60 per cent.

The average percentages of stem rust are shown in Table VIII. The range on Haynes Bluestem was from 55 to 63 per cent, and on the hybrid from 38 to 43 per cent. The differences were so small that one would be fully justified in concluding that the effect of the fertilizers on the susceptibility of the host to attacks by stem rust is negligible.

The yields were greatly reduced by the drought and the rust. There was a sharp difference in the ability of the two varieties to withstand the effects of the drought, a difference due partly at least to slightly earlier maturity of the hybrid. The average yield of Haynes Bluestem was less than a bushel per acre, while that of the hybrid was between 5 and 6 bushels per acre.

TABLE VIII.—*Summarized data on the effect of barnyard manure on the percentage of stem rust on Haynes Bluestem and on the hybrid in the plats treated with commercial plus natural fertilizer in 1916 on University Farm, St. Paul, Minn.*

Plats.	Number of plats included.	Average percentage of rust.		Range of rust percentage.	
		Bluestem.	Hybrid.	Bluestem.	Hybrid.
All . . . . .	9	58.5	40.2	55-63	34-43
Control . . . . .	1	55	38	55	38
Receiving 10 tons manure per acre.	4	60.5	41.5	58-63	40-43
Receiving 25 tons manure per acre.	4	60	41.2	58-63	40-43

## EXPERIMENTS WITH COMMERCIAL FERTILIZERS IN 1917

## GENERAL PLAN

In 1917, experiments were made on three different farms. The general plan was the same for each, but there were some minor variations. The series were as follows:

FIRST SERIES, UNIVERSITY FARM.—The plats were laid out in exactly the same place as in the previous two years. The soil therefore was the heavy Hempstead clay loam.

SECOND SERIES, QUINN FARM.—The plats were laid out on light Hempstead clay loam. They were about 1½ miles from those in the first series. The soil on the Quinn farm, while of the same general type as that on University farm, was much lighter, contained more sand and was not so well supplied with nitrogen.

THIRD SERIES, ANOKA.—The plats were laid out on Merrimac loamy sand near Anoka. The soil was very light and deficient in nitrogen.

## RESULTS OF FIRST SERIES—UNIVERSITY FARM

The experiments on University Farm were made on the same plats used in the previous two years; but acid phosphate and potassium were not applied, because it was desired to ascertain whether there would be any residual effect of these fertilizers on the plants themselves and on their resistance to stem rust. Sodium nitrate was applied as usual, as the residual effect is negligible. On April 28 one-half of each plat was sown to Marquis, C. I. 3641, which is very susceptible to stem rust in Minnesota, and the other half was sown to the same hybrid which was used in 1916.

The weather conditions in 1917 were quite different from those in 1916. The spring of 1916 was wet and cold, but soon after the plants headed it became very hot and dry. In 1917, the growing conditions generally were favorable for wheat. This is indicated clearly by the fact that there was practically no premature ripening and the yields were good in practically all of the plats. Stem rust did very little damage throughout the hard red spring wheat region and the yield of wheat was fairly satisfactory, except in North Dakota where drought reduced it. The average yield in Minnesota was 17.5 bushels per acre, in North Dakota 8 bushels per acre, and in South Dakota 14 bushels per acre (35).

The plan of the experiment and the results obtained, including the percentages of stem-rust infection on both varieties in each plat, are

given in Table IX. At first, it appears that there is a definite correlation between the amount of stem rust and the kind of fertilizer applied. The highest average percentage of rust on Marquis was on the plats which had received sodium nitrate at the rate of 1,000 pounds per acre, the next highest on those which had received 500 pounds, and the third highest, on those which had received 250 pounds. There appears, therefore, to be a direct correlation. The higher percentage of rust in these plats was due partly, at least, to delayed maturity, as the plants matured a week or 10 days later than did those in the other plats. The density of stand also no doubt made it possible for the rust to develop more abundantly, as the plants lodged badly and moisture was retained much longer than on those plants which did not lodge. (See Pl. 1, 2, and 3.)

TABLE IX.—*The disposition of the commercial fertilizers and the percentages of stem rust on Marquis and the hybrid, and the percentages of yellow-berry, seed shriveling, lodging, and the acre yields of Marquis in 1917 in the plats on University Farm, St. Paul, Minn.*

Infection, yellow-berry, seed shriveling, lodging, and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.											
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium sul- phate residual. <sup>a</sup>							
			1,000	500	250		1,000	500	250					
Percentage of stem rust, Marquis ...	Acid phosphate residual. <sup>a</sup>	Pounds.	55	33	31	33	28	29	28					
Percentage of stem rust, hybrid. ....		17	8	7	6	7	4	2						
Percentage of yellow-berry, Marquis.		0	0	50	50	80	90	90						
Percentage of seed shriveling, Mar- quis. ....		70	70	15	0	0	0	0						
Percentage of lodging, Marquis. ....		100	80	60	0	0	0	0						
Bushels per acre, Marquis. ....		12.8	14.1	28.2	35.3	24.3	21.7	22.0						
Percentage of stem rust, Marquis ...		Acid phosphate residual. <sup>a</sup>	Pounds.	60	37	35	33	32	31	26				
Percentage of stem rust, hybrid. ....				11	19	6	6	7	6	5				
Percentage of yellow-berry, Marquis.				0	0	10	75	70	80	80				
Percentage of seed shriveling, Mar- quis. ....				70	60	15	0	0	0	15				
Percentage of lodging, Marquis. ....				100	90	40	20	0	0	0				
Bushels per acre, Marquis. ....				15.8	17.5	31.8	30.5	26.9	25.9	23.9				
Percentage of stem rust, Marquis ...				Acid phosphate residual. <sup>a</sup>	Pounds.	65	37	37	32	34	31	27		
Percentage of stem rust, hybrid. ....						10	10	5	6	6	8	2		
Percentage of yellow-berry, Marquis.						0	0	5	40	70	60	90		
Percentage of seed shriveling, Mar- quis. ....						30	30	20	0	0	0	0		
Percentage of lodging, Marquis. ....						100	90	80	30	0	20	0		
Bushels per acre, Marquis. ....						18.6	20.7	26.1	32.4	30.8	35.7	32.6		
Percentage of stem rust, Marquis ...						Acid phosphate residual. <sup>a</sup>	Pounds.	67	35	38	28	32	31	25
Percentage of stem rust, hybrid. ....								9	8	4	5	7	6	1
Percentage of yellow-berry, Marquis.								0	0	5	40	60	75	70
Percentage of seed shriveling, Mar- quis. ....								40	30	15	15	0	0	0
Percentage of lodging, Marquis. ....								80	80	60	0	0	0	0
Bushels per acre, Marquis. ....								14.7	22.0	25.8	40.8	33.2	32.5	29.6

<sup>a</sup> Fertilizers applied, at rate indicated, in 1916, but not in 1917.



It will be noted that the effect of nitrogen in a year like 1917 may be quite different from that in a year like 1916. In 1916 the weather became very hot and dry shortly after the plants headed. The plants in the nitrate plats suffered more from the drought than did those in any of the other plats. The result, of course, was that the development of the rust was checked by the premature ripening of the plants. In 1917, on the other hand, the growing conditions were good, there was but little real burning out the stand in the nitrogen plats was very heavy, the individual plants were succulent, and maturity was greatly delayed, thus making conditions especially favorable for the development of stem rust.

The date of maturity often has a great influence on the degree of rust infection. It is a very commonly observed and well-known fact that early maturing varieties often escape rust, while late maturing ones may be injured severely. In certain years the rust epidemic appears just after the earlier-maturing variety has ripened, but early enough to attack the late variety while it is still susceptible. The plants in the plats heavily fertilized with nitrogen in 1917 matured several days later than those in the other plats, and the opportunity for infection was thus increased. The rust percentage on a given date was uniform in all of the plats, but the weather was still favorable for the development of rust after the earlier-maturing plants were harvested and while the later-maturing plants still were green. Naturally more rust developed on the latter. This is illustrated in Table XII with data taken from the plats on the Quinn farm.

The residual effect of the potassium and phosphate fertilizers applied to the plats prior to 1917 apparently had little or no effect on the susceptibility or resistance of the plants to stem rust. (Table IX.) The percentages of rust in the plats which had received only acid phosphate the previous year actually were slightly higher than those in the control plat. There clearly was no protective action in the potassium plats, nor in those which had received both acid phosphate and potassium sulphate. It was possible to note the residual effect of the fertilizers applied in 1916 only on one character, that of yellow-berry, which was very high in some of the plats which had received no nitrogen. The results from the seven plats receiving 2,000 pounds of acid phosphate are illustrated graphically in figure 4.

It will be seen from Table X that there was a great increase in the amount of straw on the nitrogen plats. In some cases it is almost double that on certain potassium plats. Just the reverse is true of the total weight of seed per plat. The average yield of grain for all plats receiving applications of nitrogen was 20.7 bushels per acre, while the average yield for all those not receiving nitrogen was 29.9 bushels per acre, a difference of 9.2 bushels in favor of the plats not receiving nitrogen. The ratio of weight of seed to weight of straw shows clearly the effect of the fertilizers applied to the soil on the growth of the plant. Likewise, the yields from the nitrogen plats is inversely proportional to the amount of nitrogen applied.

There appears to be little correlation between rust percentage and yield. This is especially clear when one compares the data for the plats which received 500 pounds of sodium nitrate with those for the plats which received 250 pounds (Table IX). The highest yield was obtained on the untreated control. The ratio of seed to straw for all plats receiving applications of nitrogen is 1:4.6; while the ratio for all plats not receiving applications of nitrogen is 1:2.5. The nitrogen clearly

was detrimental, but it was because it promoted excessive production of straw and low yield of grain rather than because of any effect it may have had on the rust. The highest percentage of rust was 67 in the plot which had received only sodium nitrate at the rate of 1,000 pounds during each of the three years. But this amount of rust does not necessarily cause a decrease in yield, as is shown by the fact that in 1915 the highest yield in the commercial fertilizer plots was 31 bushels per acre from a plot in which the percentage of stem rust was 88 per cent.

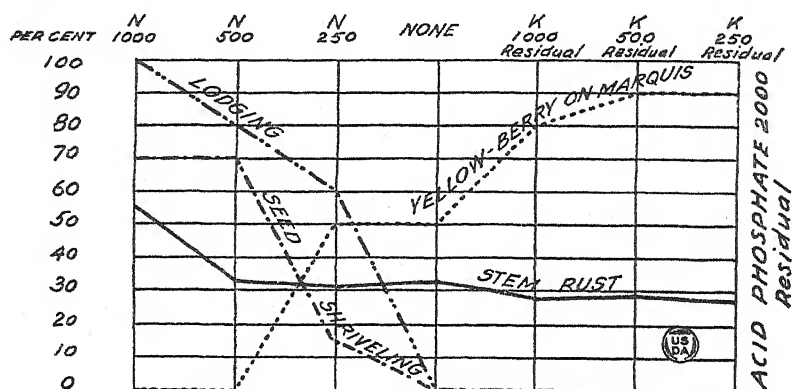


FIG. 4.—Graph showing the percentages of stem rust, yellow-berry, seed shriveling, and lodging of Marquis wheat grown in plots which had received acid phosphate at the rate of 2,000 pounds per acre and different amounts, in pounds, of potassium sulphate (K) in 1916 and different amounts of sodium nitrate (N) in 1917, in commercial fertilizer experiments in 1917 on University Farm, St. Paul, Minn.

— stem rust.  
 - - - yellow-berry.  
 . . . seed shriveling.  
 - . . lodging.

TABLE X.—The effect of fertilizers on the yields, in kilograms, of seed and straw of Marquis wheat and on the ratio of seed to straw in the commercial fertilizer plots in 1917 on University Farm, St. Paul, Minn.

Weight of seed and straw, and ratio of seed to straw.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.						
	Kind.	Amount per acre.	Sodium nitrate.			None.	Potassium chlorid residual. <sup>a</sup>		
			1,000	500	250		1,000	500	250
		Pounds.							
Weight of seed. ....	Acid phosphate residual. <sup>a</sup>	2,000	1.1	1.3	2.5	3.2	2.2	1.9	2.0
Weight of straw. ....			7.7	8.4	7.9	7.4	5.3	5.6	4.7
Ratio, seed to straw...			1:7	1:6.4	1:3.2	1:2.3	1:2.4	1:3	1:2.4
Weight of seed. ....	Acid phosphate residual. <sup>a</sup>	1,000	1.4	1.6	2.8	2.8	2.4	2.3	2.1
Weight of straw. ....			8.4	7.0	7.8	7.5	6.0	5.3	4.6
Ratio, seed to straw...			1:6	1:4.4	1:2.8	1:2.7	1:2.5	1:2.3	1:2.2
Weight of seed. ....	Acid phosphate residual. <sup>a</sup>	500	1.7	1.9	2.3	2.9	2.8	3.2	2.9
Weight of straw. ....			9.1	7.7	9.2	9.5	7.6	7.5	7.3
Ratio, seed to straw...			1:5.4	1:4.1	1:4	1:3.3	1:2.7	1:2.3	1:2.5
Weight of seed. ....	Acid phosphate residual. <sup>a</sup>	None.	1.3	2.0	2.3	3.6	3.0	2.9	2.6
Weight of straw. ....			8.7	7.8	8.0	7.5	6.6	7.1	7.1
Ratio, seed to straw...			1:6.7	1:3.9	1:3.5	1:2.1	1:2.2	1:2.4	1:2.7

<sup>a</sup> Fertilizers applied, at rate indicated, in 1916, but not in 1917.

## RESULTS FROM SECOND SERIES—QUINN FARM

The second series was laid out on rather light, Hempstead clay-loam soil and consisted of 32 plats. The general plan was similar to that followed on University Farm, except that on the Quinn farm certain combinations were made between sodium nitrate and potassium chlorid, and other combinations of sodium nitrate, potassium chlorid and acid phosphate. The plats were sown to Marquis and the hybrid, as in the first series. The disposition of the plats, the rate and kind of fertilizer applied to each, the percentage of stem rust on both varieties, and the yield for Marquis are given in Table XI.

TABLE XI.—*The disposition of the fertilizers applied, the percentages of stem rust on Marquis and the hybrid, and the yields in bushels per acre of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Infection and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.	
			1,000	500	250				500	500
		<i>Pounds.</i>								
Percentage of stem rust, Marquis	Acid phosphate.	2,000	{80	28	12	5	6—	5—	10	9
Percentage of stem rust, hybrid.			{20	10	2	1	2	1	1	1
Bushels per acre, Marquis. ....			{24.2	43.9	27.6	14.3	25.1	22.3	20.2	20.0
Percentage of stem rust, Marquis		1,000	{76	65	9	8	8	8	12	11
Percentage of stem rust, hybrid.			{20	8	3	1	1	1	2	2
Bushels per acre, Marquis. ....			{33.4	29.6	33.6	30.2	30.0	31.7	42.9	38.1
Percentage of stem rust, Marquis		500	{10	9	8	7	7	7	10	11
Percentage of stem rust, hybrid.			{3	1	1	1	1	1	5	3
Bushels per acre, Marquis. ....			{42.7	37.8	29.0	21.9	23.6	23.2	40.5	39.6
Percentage of stem rust, Marquis		None.	{11	11	10	10	10	10	27	18
Percentage of stem rust, hybrid.	{4		1	1	1	1	2	20	22	
Bushels per acre, Marquis. ....	{33.7		42.7	37.4	29.0	30.1	28.4	37.6	16.4	
							500	250	500	250
							Potassium chlorid.			
							Fertilizer, kind and amount (pounds) per acre.			

It was pointed out in the discussion of results on University Farm that the increased percentage of rust on a few of the plats to which sodium nitrate had been applied was due to delayed maturity and not to an increased degree of susceptibility. This fact is illustrated clearly in Table XII. Even with the slight difference in these plats, it is apparent that in these experiments the effect of the fertilizers on the susceptibility of the wheat to attacks by stem rust is too small to be significant.

The percentages of stem rust for the various plats in the column headed "Percentage of rust, August 10," it will be noted, are very uniform. For the hybrid they run, 1, 2, 2, 5, 2, 4, and 1 per cent, respectively, for plats 22, 30, 23, 31, 24, 32, and 28, respectively. This same series ten days

later, however, as shown in column headed "Percentage of rust August 20," is far from uniform. For the hybrid, they run 1, 2, 5, 20, 3, 22, and 1 per cent, respectively, for the same plats. When compared with the column giving the number of days from planting to maturity, it will be noted that there is a direct correlation between the dates of maturity and the percentages of stem rust. It will be noted further that it was only on the plats fertilized with sodium nitrate that there was sufficient delay in maturity to result in a higher percentage of stem rust.

TABLE XII.—*The effect of fertilizers on the date of maturity and on the percentages of stem rust on Marquis and the hybrid in 1917 on the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Plat No.	Kind and amount of fertilizer <sup>a</sup> in pounds per acre.	Number of days from planting to maturity.		Percentage of stem rust.			
				Aug. 10 (100 days after sowing).		Aug. 20 (110 days after sowing).	
		Marquis.	Hybrid.	Marquis.	Hybrid.	Marquis.	Hybrid.
22	{ K 250.....	100	101	7	1	7	1
	{ P 500.....						
30	{ K 250.....	102	103	10	2	10	2
	{ P 500.....						
23	{ N 500.....	104	105	9	2	10+	5+
	{ P 500.....						
31	{ K 500.....	108	110	14	5	27	20
	{ N 500.....						
24	{ K 250.....	104	105	9	2	11	3+
	{ N 500.....						
	{ P 500.....	108	111	12	4	18	22+
32	{ K 250.....						
	{ N 500.....	102	104	10	1	10	1
28	{ Control.....						

<sup>a</sup> N=sodium nitrate; K=potassium chlorid; P=acid phosphate.

The percentages of stem rust are high in four of the plats in the upper left-hand corner of Table XI. Unfortunately, these plats were in the shadow of a barn during part of the day, so the plants remained wet longer after dews and rains than did those in any of the other plats. It was clearly evident that this shading and late maturity were chiefly responsible for the increased percentage of rust in this part of the field.

The fertilizers affected the general growth of these plants more than those on University Farm. The average height of the plants in all plats which received applications of sodium nitrate was 40.3 inches, while the average height of the plants in all other plats was 36.25 inches. These data, with others on weight of seed and of straw, and the ratio of seed to straw, are given in Table XIII.

TABLE XIII.—The effect of fertilizers on the height of plants in inches; on the yield, in kilograms, of seed and straw; and on the ratio of seed to straw, of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.

	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
Straw height, seed and straw weight, and ratio of seed to straw.	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.	
			1,000	500	250				500	500
Acid phosphate.										
		Pounds.								
Height of straw.....	}	2, 000	40	44	44	35	36	32	42	40
Weight of seed.....			2.2	3.9	2.5	1.3	2.2	2.0	3.6	3.6
Weight of straw.....			12.8	13.7	11.0	6.5	6.5	5.3	11.7	11.5
Ratio, seed to straw....			1:5.8	1:3.5	1:4.4	1:5	1:3	1:2.7	1:3.3	1:3.2
Height of straw.....	}	1, 000	39	39	45	39	39	38	42	41
Weight of seed.....			3.0	2.6	3.0	2.7	2.7	2.8	3.9	3.4
Weight of straw.....			9.9	13.8	12.4	8.1	8.4	8.4	12.8	12.4
Ratio, seed to straw....			1:3.3	1:5.3	1:4.1	1:3	1:3.1	1:3	1:3.3	1:3.6
Height of straw.....	}	500	40	40	36	35	35	33	36	38
Weight of seed.....			3.8	3.4	2.6	1.9	2.1	2.1	3.6	3.5
Weight of straw.....			12.1	10.3	8.4	5.6	6.2	5.9	11.1	11.2
Ratio, seed to straw....			1:3.2	1:3	1:3.2	1:2.9	1:3	1:2.8	1:3.1	1:3.2
Height of straw.....	}	None.	38	39	40	38	38	37	37	37
Weight of seed.....			3.0	3.8	3.3	2.6	2.7	2.5	3.4	1.5
Weight of straw.....			10.8	11.5	10.5	7.3	7.4	7.1	11.1	11.2
Ratio, seed to straw....			1:3.6	1:3	1:3.2	1:2.8	1:2.7	1:2.8	1:3.2	1:7.5
							500	250	500	250
Potassium chlorid.										
Fertilizer, kind and amount (pounds) per acre.										

The plants in the plats fertilized with sodium nitrate tillered better, were more vigorous, and yielded better than those on the plats which had received no nitrogen. The average yield from the nitrate plats was 35.8 bushels per acre, while that from those receiving no nitrogen was 25.8 bushels, a difference of ten bushels in favor of the plats fertilized with nitrogen. The ratios of seed to straw were quite uniform. The effect of sodium nitrate on yield was quite different from that in the plats on University Farm. Here there was a difference of 9.2 bushels in favor of the plats not receiving nitrogen, while on the Quinn farm there was a difference of 10 bushels in favor of the plats receiving nitrogen. Evidently the soil on the Quinn farm was deficient in available nitrogen, while that on University Farm was not.

The data in Table XIV show also the correlation between the fertilizers applied and the percentages of "yellow-berry" and protein in the seed. The average percentages of yellow-berry and protein content of the seed for all plats which received applications of sodium nitrate are 14.5 per cent and 14.7 per cent, respectively, compared with 67 per cent and 12.8 per cent, respectively, as the averages for all plats which received no

applications of sodium nitrate.<sup>10</sup> Heavy applications of nitrogen caused some lodging and shriveling of the seed.

TABLE XIV.—*The effect of fertilizers on the percentages of yellow-berry, protein in seed, seed shriveling, and lodging of Marquis in 1917 in the commercial fertilizer plats on the Quinn farm, St. Paul, Minn.*

Yellow-berry, protein in seed, seed shriveling, and lodging.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.							
	Kind.	Amount per acre.	Sodium nitrate.				None.		Sodium nitrate.	
			1,000	500	250				500	500
		Pounds.								
Percentage of yellow-berry....	Acid phosphate.	2,000	0	20	80	70	85	85	25	10
Percentage of protein in seed <sup>a</sup> ...			16.6	15.1	13.8	12.9	13.3	13.6	14.3	14.4
Percentage of seed shriveling...			30	0	0	0	0	0	0	20
Percentage of lodging.....			80	30	0	0	0	0	20	20
Percentage of yellow-berry....		1,000	1	5	45	80	85	70	5	1
Percentage of protein in seed <sup>a</sup> ...			16.4	15.9	14.5	12.6	11.7	12.7	14.9	15.5
Percentage of seed shriveling...			30	20	0	20	0	0	20	20
Percentage of lodging.....			40	70	20	0	0	0	30	40
Percentage of yellow-berry....		500	0	10	50	50	70	60	15	8
Percentage of protein in seed <sup>a</sup> ...			16.3	14.5	13.4	13.2	12.4	12.6	13.6	14.5
Percentage of seed shriveling...			0	0	0	0	0	0	20	20
Percentage of lodging.....			30	0	0	0	0	0	20	20
Percentage of yellow-berry....		None.	0	2	5	50	50	50	2	5
Percentage of protein in seed <sup>a</sup> ...			15.9	14.8	14.0	13.1	12.4	13.1	13.9	14.0
Percentage of seed shriveling...			30	20	0	0	0	0	20	0
Percentage of lodging.....			50	0	0	0	0	0	30	20
							500	250	500	250
							Potassium chlorid.			
							Fertilizer, kind and amount (pounds) per acre.			

<sup>a</sup> On dry basis.

From the data obtained, it is very evident that the normal physiology of the plants was changed considerably on the different plats. This is shown both by observations on the morphology of the plants and by chemical analysis, but these changes apparently affected stem rust only indirectly by affecting density of stand and time of ripening.

#### RESULTS FROM THIRD SERIES—ANOKA

The third series was laid out on a light, loamy sand near Anoka, Minn. The plan of the plats was similar to that of the second series, both in disposition of the plats and in the application of fertilizers. The full square rod was sown to the one variety, Marquis, instead of two varieties as in the preceding experiments. The disposition of the plats, the rate and

<sup>10</sup> These analyses were made by Dr. C. H. Bailey, in charge of the section of cereal technology of the division of agricultural biochemistry of the University of Minnesota.

kind of fertilizer applied to each, the percentages of stem rust, yellow-berry and seed shriveling, and the yield in bushels per acre are given in Table XV.

TABLE XV.—The disposition of the commercial fertilizer plats and the effect of the fertilizers on the percentages of stem rust, yellow-berry and seed shriveling, and on yield in bushels per acre for Marquis wheat, at Anoka, Minn., in 1917

Infection, yellow-berry, seed shriveling and yield.	Fertilizer.		Fertilizer, kind and amount (pounds) per acre.								
	Kind.	Amount per acre.	Sodium nitrate.			None.			Sodium nitrate.		
			1,000	500	250				500	500	
		Acid phosphate.	Pounds.	15	11	10	11	8	9	14	13
Percentage of stem rust . . . . .			2,000	45	50	70	65	95	60	25	75
Percentage of yellow-berry . . . . .				30	20	25	15	0	0	40	15
Percentage of seed shriveling . . . . .				24.1	33.3	23.2	19.5	22.2	23.0	29.3	25.1
Bushels per acre . . . . .											
Percentage of stem rust . . . . .			1,000	15	11	12	8	15	12	15	18
Percentage of yellow-berry . . . . .				30	60	90	90	35	60	40	40
Percentage of seed shriveling . . . . .				15	15	15	0	0	20	20	15
Bushels per acre . . . . .				41.7	20.1	24.4	14.9	15.2	14.0	32.0	31.4
Percentage of stem rust . . . . .			500	17	13	10	15	12	12	14	15
Percentage of yellow-berry . . . . .				15	75	98	95	90	60	75	75
Percentage of seed shriveling . . . . .				40	20	0	0	0	0	15	20
Bushels per acre . . . . .		23.8		28.3	27.3	14.5	15.1	14.8	34.8	28.0	
Percentage of stem rust . . . . .		None.	16	17	16	11	13	9	15	16	
Percentage of yellow-berry . . . . .			0	70	90	95	95	75	55	75	
Percentage of seed shriveling . . . . .			0	30	0	0	0	0	0	15	
Bushels per acre . . . . .			13.4	22.4	23.1	13.6	12.2	18.3	27.5	33.8	
							500	250	500	250	
							Potassium chlorid.				
							Fertilizer, kind and amount (pounds) per acre.				

The percentages of stem rust in these plats at Anoka varied but little (Table XV). The percentages of yellow-berry and seed shriveling were affected by fertilizers about as they were in the preceding experiments. The yields were increased by nitrogen much as they were on the Quinn farm. The average yield for all plats receiving applications of sodium nitrate was 27.4 bushels per acre, and for all plats receiving none, it was 16.4 bushels, a difference of 11 bushels in favor of the plats receiving nitrogen. The data obtained from this experiment tend to show that stem rust was affected but little, even though the morphology and physiology of the wheat plants were affected considerably by fertilizers.

### GENERAL OBSERVATIONS, 1918 TO 1922

After 1917, observations were made on permanent tenth-acre triplicated plats of the division of soils of the University of Minnesota, at the Crookston substation, and observations were made one year on similar

plats at the Morris substation. The description of the soil at Crookston is given in the discussion of methods. Each plat was sown to Marquis wheat and no attempt was made to produce artificial epidemics of stem rust. Notes on stem rust were taken just before the plants ripened.

The results for 1918 and 1920 are given in Table XVI. Detailed figures are not available for 1919 and 1921. However, there was little observable effect of fertilizers on the development of stem rust. At Morris, in 1921, the wheat in the phosphate plats grew more vigorously and produced a denser stand than that on the other plats. But this seemed to have but little effect on the amount of stem rust, due partly to early maturity. The stand on manured plats was heavy, but the plants ripened so late that there was a slight increase of stem rust.

The differences between rust percentages in various plats in 1918 are not great (Table XVI). When the averages for the three replications are made, the differences are not significant. There is a complete lack of correlation between the percentage of stem rust on the plants and the fertilizers applied to the soil.

TABLE XVI.—*The percentages of stem rust on Marquis in 1918 and 1920 in fertilizer plats at Crookston, Minn.*

Plat No.	Fertilizer per acre.	Percentage of stem rust.	
		1918.	1920.
1	Control.....	40	50
2	1 ton rock phosphate.....	30	50
3	{ 8 tons manure.....	35	50
4	{ 1 ton rock phosphate.....		
5	8 tons manure.....	50	40
6	{ 8 tons manure.....	35	50
7	{ 480 pounds acid phosphate.....		
8	480 pounds acid phosphate.....	35	50
9	Control.....	47	70
10	1 ton rock phosphate.....	50	60
11	{ 8 tons manure.....	50	15
12	{ 1 ton rock phosphate.....		
13	8 tons manure.....	45	25
14	{ 8 tons manure.....	40	50
15	{ 480 pounds acid phosphate.....		
16	480 pounds acid phosphate.....	45	30
17	Control.....	30	50
18	1 ton rock phosphate.....	45	30
19	{ 8 tons manure.....	40	20
20	{ 1 ton rock phosphate.....		
21	8 tons manure.....	45	40
22	{ 8 tons manure.....	50	50
23	{ 480 pounds acid phosphate.....		
24	480 pounds acid phosphate.....	40	25

In 1921, there was a wide variation in percentage of stem rust in certain individual plats, but in the three replications these differences were not consistent, a fact attributed to the very spotted and nonuniform occurrence of the rust, so characteristic of the epidemic throughout the State. Early in July drought checked the spread of rust, and a uniform epidemic



prior to the maturity of the plants could not be expected. The average percentages of stem rust for the three replications are fairly uniform and show no consistent correlations with the fertilizers. A few more plat replications undoubtedly would have made the results just as conclusive as those for 1918.

In 1922, observations were made on a field of winter wheat at the Crookston substation in which several strips across the field had been fertilized with acid phosphate. No difference in growth or maturity of the plants could be observed and there was a perfectly uniform stem rust infection of 10 per cent on all the plats. Observations were made also on plats of Mindum, C. I. No. 5296, a durum wheat, in a four-year rotation series with different rates of manuring. The results are given in Table XVII. The amount of lodging was directly proportional to the amount of manure applied, but the degree of stem rust infection was uniform in all of the plats, at least as late as about two weeks before harvest.

TABLE XVII.—*The effect of barnyard manure on degree of lodging and percentages of stem rust on Mindum in 1922 in the fertilizer plats at Crookston, Minn.*

Amount of manure.	Percentage of plants lodged.	Percentage of stem rust.
None.....	0	10
4 tons.....	Trace.	10
8 tons.....	5	10
16 tons.....	25	10
16 tons (in 2 applications).....	35	10
32 tons.....	60	10

#### SIGNIFICANCE OF RESULTS

The average percentage of rust in all plats receiving nitrogen compared with the average percentage in those receiving none gives a fair indication of the resultant differences from the two treatments. The averages, however, may or may not be a true expression of the differences between the two series of plats. If the individual percentages entering into the average are relatively small, it becomes a fairly accurate expression, but, as these increase, the average becomes less and less a reliable expression of the true condition. In considering such a variable as the effect of fertilizers, comparison of two series in an experiment comprising a small number of plats is most availably presented by the method given by Student in *Biometrika* (34). By this method the resulting differences between the treatments are expressed in odds.

In the following discussion of results, the odds have been calculated mainly for the rust percentages and yield, these being the two chief characters under consideration in this paper. The odds are calculated from the results on two adjacent series of plats. The series of four plats receiving 250 pounds of sodium nitrate were adjacent to a series of four receiving none. These two series of homologous plats are used in all of the experiments with commercial fertilizers. They are shown in Table I as plats 3, 10, 17, and 24 for the nitrogen series; and plats 4, 11, 18, and 25 for the adjacent non-nitrogen series. In the case of manured plats in the years 1918 and 1920 (Table XVII) the odds are calculated by comparing

the results from the manured plats with those from the plats which received no manure.

From a general inspection of the data obtained in 1915 (Table III), one can readily determine that the difference in rust percentages on the two series of plats are not great enough to be considered significant. This fact is further demonstrated by the calculated odds of only 4.1:1 for Haynes Bluestem in favor of the nitrogen plats. Mathematicians have shown that in order to be significantly different the odds in favor of one treatment over another ought to be 30:1, which is approximately 3.2 times the probable error. With Iumillo, the odds for rust percentages are 7.4:1 in favor of the plats not receiving nitrogen. The differences obtained on these plats in this experiment, therefore, are not significant, and the only possible conclusion that can be drawn is that the differences in rust percentage caused by the two treatments are negligible. In the case of yield of grain of Haynes Bluestem, the odds are 42.5:1 in favor of the series not receiving nitrogen, indicating a significant difference for this character. The average acre yield for all plats receiving nitrogen is 12.6 bushels, and for all others it is 23 bushels.

In 1916 (Table VI) the calculated odds for stem rust on Haynes Bluestem are 9.2 : 1 in favor of the series not receiving applications of nitrogen, and therefore are not significant. With the hybrid, the odds are 55.5 : 1 in favor of the plats not receiving applications of nitrogen. These odds indicate a significant difference. The average rust percentage on the hybrid, however, for all plats receiving nitrogen and for those not receiving nitrogen are 47.9 and 43.3 per cent, respectively. The calculation of the odds for these two series appears to give a significant difference, but when considered with the average rust percentages for the experiment as a whole, the results are not so convincing. In the case of yield of grain of Haynes Bluestem, the odds are 28.9 : 1 in favor of the series not receiving nitrogen, indicating a fairly significant difference for this character. The average acre yield for all plats receiving nitrogen is 0.77 of a bushel and for all others it is 2.9 bushels.

In the experiment at University Farm in 1917 (Table IX) the calculated odds for rust on Marquis are 7.5 : 1 in favor of the series receiving applications of nitrogen. For the hybrid, the odds are only 2 : 1 in favor of the series not receiving applications of nitrogen. It is evident that the differences in rust percentages on either variety in the two series are not large enough to be significant. In the case of yield of grain of Marquis, the odds are only 11.6 : 1 in favor of the series not receiving nitrogen, and therefore are not significant. The average acre yields for all nitrogen plats is 20.7 bushels and for all others it is 29.9 bushels.

On the plats at the Quinn farm in 1917 (Table XI), the calculated odds for rust on Marquis are 6.8 : 1 in favor of the series receiving nitrogen. For the hybrid, the odds are 8.2 : 1 in favor of the series receiving nitrogen. These odds are too small to be considered significant for either variety. In the case of yield of grain of Marquis the odds are 67 : 1 in favor of the series receiving nitrogen, a very significant difference. The average acre yield for all nitrogen plats is 33.5 bushels and for all other plats is 25.8 bushels. This experiment was made on a rather light soil and the yields were increased by applications of nitrogen, contrasting strongly with the results of the preceding experiments which were on a rather heavy soil on which nitrogen decreased yields.

The calculated odds for height of plants of Marquis are 20.7 : 1 in favor of the series receiving nitrogen. (Table XIII.) These odds are hardly great enough to be considered significant. The differences between the individual plats, however, are quite consistent, and the average height of plants on the plats receiving nitrogen is 39.8 inches and for all others it is 36.2 inches.

The calculated odds for percentage of crude protein (Table XIV) in the seed of Marquis is 21.5 : 1 in favor of the series receiving nitrogen. These are only fairly significant; but here, too, as for height of plants, the differences between the individual plats are small but quite consistent. The average percentage of crude protein for all plats receiving nitrogen is 15.8 compared with 12.9 per cent for all other plats.

On the plats at the Anoka farm in 1917 (Table XV) the calculated odds for rust on Marquis are only 1.6 : 1 in favor of the series receiving nitrogen. For yield of grain, however, the odds are 45.3 : 1 in favor of the series receiving nitrogen. This experiment was made on a light, sandy soil, and the application of sodium nitrate was very beneficial. The average yield per acre for all plats receiving nitrogen is 27.4 bushels and that of all other plats is 16.4 bushels.

Unfortunately, the experiments in 1915 and 1916 with barnyard manure were not laid out so that it would be feasible to arrange the plats in series and calculate odds for significance of difference in results. In 1915 the average percentages of rust for Bluestem and the hybrid on the manured plats was 83 and 38.4 per cent, respectively, compared to 70 and 33 per cent, respectively, for the controls. These rust percentages, which were taken at the time of maturity of the last plat to ripen, appear to indicate significant differences. There is a direct relation to the time of maturity, however, as is illustrated in Tables IX and XVII where, on a given day, say the date of maturity of the first control, there are no differences in the rust percentages in the various plats, but where some of the plats remain green and exposed to infection for a longer period of time, their total percentage of rust infection is bound to increase. In 1915 the average number of days from seeding to maturity was 114.6 compared to the controls with an average of 102.7.

#### GENERAL CONCLUSIONS

In discussing the effect of fertilizers on the susceptibility of wheats to *Puccinia graminis*, it is necessary to consider what is meant by this susceptibility. Plants may be physiologically resistant, or morphologically resistant, or they may merely be disease-escaping. That there is a true physiologic or protoplasmic basis for resistance to *P. graminis*, probably none will deny. It is well known that this is a genetic character comparable with other hereditary characters. The results of field experiments and observations suggest also that plants sometimes may be resistant on account of morphologic characters. Certainly, plants with a large amount of woody tissue are not likely to be so severely rusted as are those with a larger proportion of succulent tissue because the rust fungus requires the latter for growth. But this morphologic resistance is quite different from true protoplasmic resistance; it is only a mechanical limitation on the spread of the rust mycelium. Under normal conditions, in some varieties, it undoubtedly is due to inherited genetic factors, while in other varieties it may be due to environmental conditions. It also is well known that early maturing varieties may ripen

early enough to escape infection to some extent. The differences between these kinds of resistance, on the one hand, and those phenomena on the other hand which clearly can not be defined as resistance, should be kept distinctly in mind, as they probably have been confused frequently in field studies similar to those reported in this paper. This confusion, together with the fact that opportunities for infection often are affected considerably by density of stand and other factors, has given rise to the view that resistance to rust can be modified easily by changing environmental conditions.

Resistance of wheat varieties to *Puccinia graminis* may not be rigidly immutable. One would not expect it to be. As plant characters are the resultant of the interaction of genetic and environmental factors, fluctuations are bound to occur. Otherwise every plant of the same pure line should always be exactly like all the other plants of that pure line. But such is not the case. Neither are all of the individuals of a pure-line wheat variety affected equally severely by stem rust, even when they are growing under apparently identical environmental conditions. Yet, two plants seldom if ever grow under identical conditions. It is necessary, therefore, to distinguish as clearly as possible between the effects of genetic factors and those of environmental factors. The results presented in this paper lend practically no support to the opinion that the genetic resistance of wheat varieties is changed by fertilizers. The type of infection remained the same on susceptible varieties<sup>11</sup> like Glyn-don Fife, Haynes Bluestem, and Marquis and on resistant varieties like Iumillo and the resistant hybrids, regardless of fertilization. More and larger uredinia sometimes developed on wheat in some fertilizer plats than on that in others. This seemed to be due, however, to increased opportunity for infection or to structural changes in the plants which made it possible for the rust mycelium to spread more extensively.

Morphologic resistance may be changed somewhat by fertilizers. It is clear from the data obtained that the height of the plants and stiffness of straw are affected greatly by different fertilizers. The stiffness of straw, of course, is an index of the amount of supportive tissue in the plant. *P. graminis* and other cereal-rust fungi grow almost entirely in chlorenchymatous tissues. If the fertilization is such as to increase the amount of mechanical tissue, the rust mycelium can not thrive as well as it can in plants containing large amounts of chlorenchyma. But the writers found little evidence that, under field conditions, anything approaching normal fertilization changed the morphologic resistance to stem rust greatly. There was much more convincing evidence that fertilizers affected the final degree of rustiness of plants by hastening or delaying ripening and by affecting the stand.

The degree of infection by orange leaf rust seemed to be increased by heavy applications of nitrogen in some plats, but the rust was not abundant enough in most of them to make it possible to draw final conclusions.

Any fertilizers which prolong the growing period and thus delay maturity are likely to increase indirectly the amount of rust. Late maturing plants are exposed to inoculation longer than are those which mature early. A few days' difference between the dates of ripening of two fields of wheat may have a marked influence on the amount of rust which

<sup>11</sup> This statement applies to susceptibility to the biologic forms used and to the general reaction of the varieties in the field at University Farm, St. Paul.

may develop. In the hard red spring wheat region the stem rust epidemic is likely to come just before harvest. Early ripening fields often escape great damage, whereas those which mature a week later may be damaged severely. Under fairly normal growth conditions for wheat, barnyard manure and other nitrogenous fertilizers may delay ripening for a few days or even for two weeks or more, depending on the soil type and amount of fertilizer used. It has been shown that the percentage of stem rust often could be correlated directly with the time required for maturity. The conclusion is that there is more rust on the late-ripening wheat than on that which ripens earlier. For practical purposes, therefore, nitrogen has been conducive to rust, but it has not increased susceptibility in the strict sense; it has only prolonged the growth period so as to expose the plants longer to the rust danger. But the effect may be just the opposite. In 1916, for instance, when the weather was very hot and dry after the wheat headed, the plants in the nitrogen plats actually ripened earlier than those in the other plats. It is true that it was not normal ripening, the plants being practically "burned up," but the natural result was that the rust was checked also.

Fertilizers also may make it possible for the rust fungus to infect plants more easily. When nitrogenous fertilizers increase the density of stand, succulence of the plants and consequent lodging, moisture is retained longer in the field than in those in which the stand is not so dense and the plants remain upright. The minimum incubation period for the uredinal stage usually is about six hours. There is an increase in the number of germ tubes which enter the plants as the incubation period is increased. Assuming that two fields have been inoculated equally heavily, other things being equal, the heavier infection will occur in the field in which moisture is retained longer.

It is perfectly clear from the data obtained that the percentage of stem rust on wheat plants does not necessarily indicate the amount of damage which will be done. When wheat is grown in properly fertilized soil it often yields well in spite of heavy attacks of rust, while that growing in soil with unbalanced fertilization may yield poorly regardless of the degree of severity of rust. It seems very likely, therefore, that much of the damage often attributed to rust when wheat is growing in soil containing an overabundance of nitrogen, actually is due to the direct effect of the fertilizer on the plants. It is quite likely also that a given amount of rust may injure plants too heavily fertilized with nitrogen more than it does plants growing in soil containing well balanced soil nutrients. This may be due partly to increased water requirement, still further increased by rust infection, and partly to weak straw which may be weakened still more by rust. The rational thing to do is to supply the soil with the nutrients which the plants need. Nitrogen decreased yields consistently on University Farm soils, but on the lighter soils on the Quinn farm and at Anoka it increased them.

The final conclusion seems to be justified that wheat can not be predisposed easily to *Puccinia graminis*, if we mean by predisposition the action of environmental factors in rendering a variety more susceptible than it normally would be. Nitrogen apparently may predispose plants by increasing succulence, density of stand and lodging, by delaying maturity and by decreasing yields on account of these factors, and sometimes by premature ripening in hot, dry weather. But the actual predisposing action apparently is of relatively minor importance. Since the rust fungi are highly specialized obligate parasites, a mere weakening of the

host not only does not usually increase susceptibility, but may decrease it. The relationship between host and parasite is so intimate that it is difficult to generalize about it. As a rule, however, plants which grow normally are likely to yield best, regardless of rust. And the yield must be the real test of the value of fertilizers.

For practical purposes much can be done to increase yields when stem rust is prevalent. The old principle of avoiding excessive applications of nitrogenous fertilizers is sound. But some soils need them and on such soils wheat will yield better when they are supplied. Acid phosphate and potassium often are valuable in increasing stiffness of straw and promoting early maturity. It is useless to add them, however, to soils which do not need them, or in which they do not become available. The opinion that they have an actual immunizing effect against stem rust does not seem to be justified, although the indirect effect may be very great. The problem of fertilization, in order to reduce losses from rust, varies with soil type and climate. The writers are forced to the conclusion that, while the direct effect of fertilizers on the susceptibility of wheat to stem rust under field conditions is slight, the indirect effect on rust and the direct effect on yield may be very great. Give the soil those fertilizers which the wheat needs, but avoid too much nitrogen, add potash and phosphates judiciously, and the best results are likely to be obtained.

#### SUMMARY

Experiments and observations, covering a period of eight years, have been made to determine the effect of artificial and natural fertilizers on the amount of stem rust developed on susceptible and resistant varieties of wheat, when grown on several soil types in different parts of Minnesota. The plants were grown under heavy artificially induced epidemics, and under natural field epidemics.

The effect of barnyard manure, clover soil, alfalfa soil, and cabbage soil, with and without fertilizers, was tried in the field. In addition, various amounts of sodium nitrate, acid phosphate, and potassium chlorid or potassium sulphate, alone and in combinations with each other, were added to soils to determine the effect on infection of wheat by rust.

The degree of physiologic susceptibility of susceptible and resistant varieties apparently was not changed directly by the use of different fertilizers, although morphologic resistance may be changed slightly.

Plants growing in plats heavily fertilized with nitrogenous manures sometimes were more heavily attacked by stem rust than those in other plats. This apparently was an indirect effect due to increased density of stand and delayed maturity, which made conditions for infection more favorable and lengthened the time during which the plants could become infected. In hot, dry weather, plants heavily fertilized with nitrogen may burn out, thus indirectly causing a decrease in the amount of rust.

There is some evidence that the amount of orange leaf rust may be increased by the use of nitrogenous fertilizers.

The direct effect of fertilizers on the character of plant growth and yields seems to be much more important than their effect on the severity of stem rust.

The date of maturity, degree of lodging, crinkling, shriveling of seed, percentage of yellow-berry, and yield of straw and grain may be affected profoundly by different fertilizers.

On some soils, and under certain weather conditions, heavy fertilization with nitrogen decreases yield greatly by increasing the proportion of straw to seed, by inducing lodging and burning out. This probably has been confused with rust injury.

On soils deficient in nitrogen, barnyard manure and nitrates increased yields without increasing the severity of attack by stem rust.

Neither acid phosphate nor potassium counteracted the harmful effects of excessive fertilization with nitrogen on some soils.

On properly fertilized soil wheat yielded well in spite of heavy attacks of stem rust.

While the direct effect of fertilizers on the development of stem rust seems to be slight, there is sometimes a profound indirect effect. It is advisable, therefore, to avoid excessive fertilization with nitrogen and to use phosphates and potassium fertilizers on those soils which need them. Rust damage can be reduced in this way.

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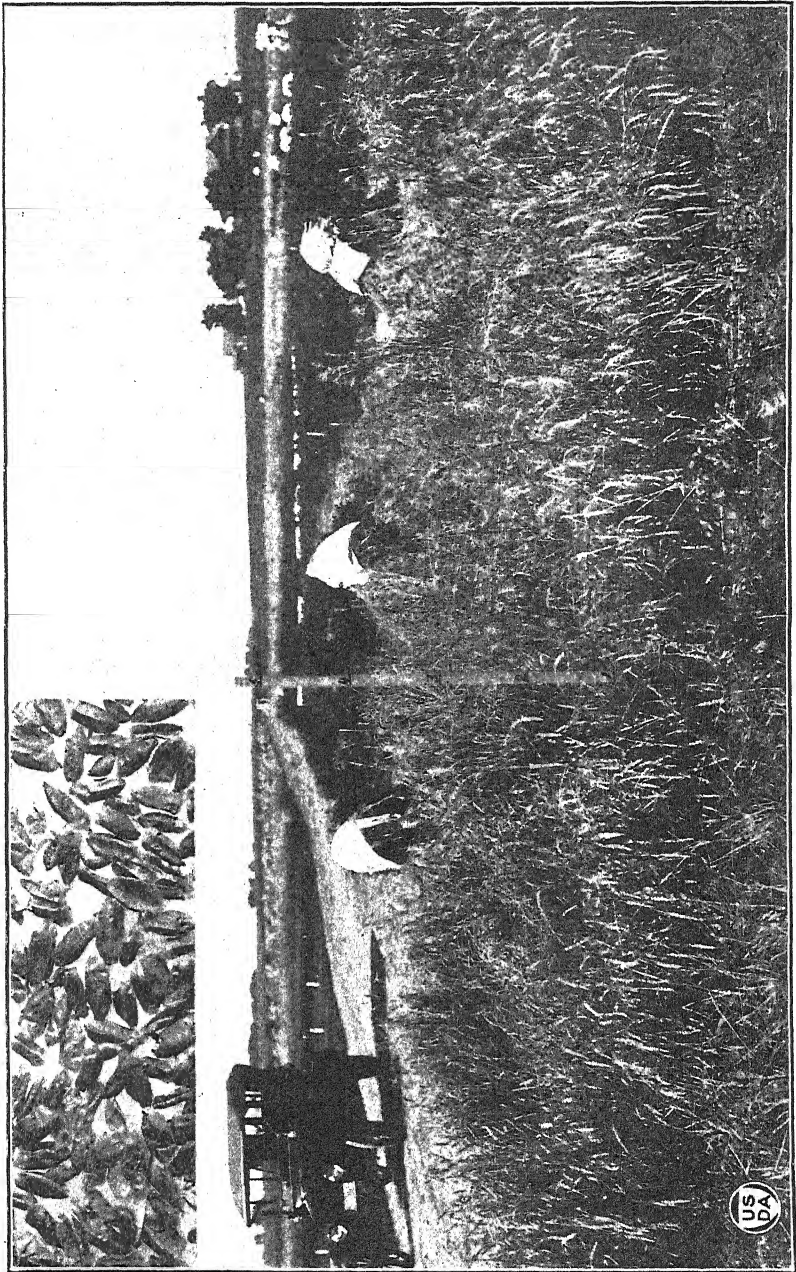
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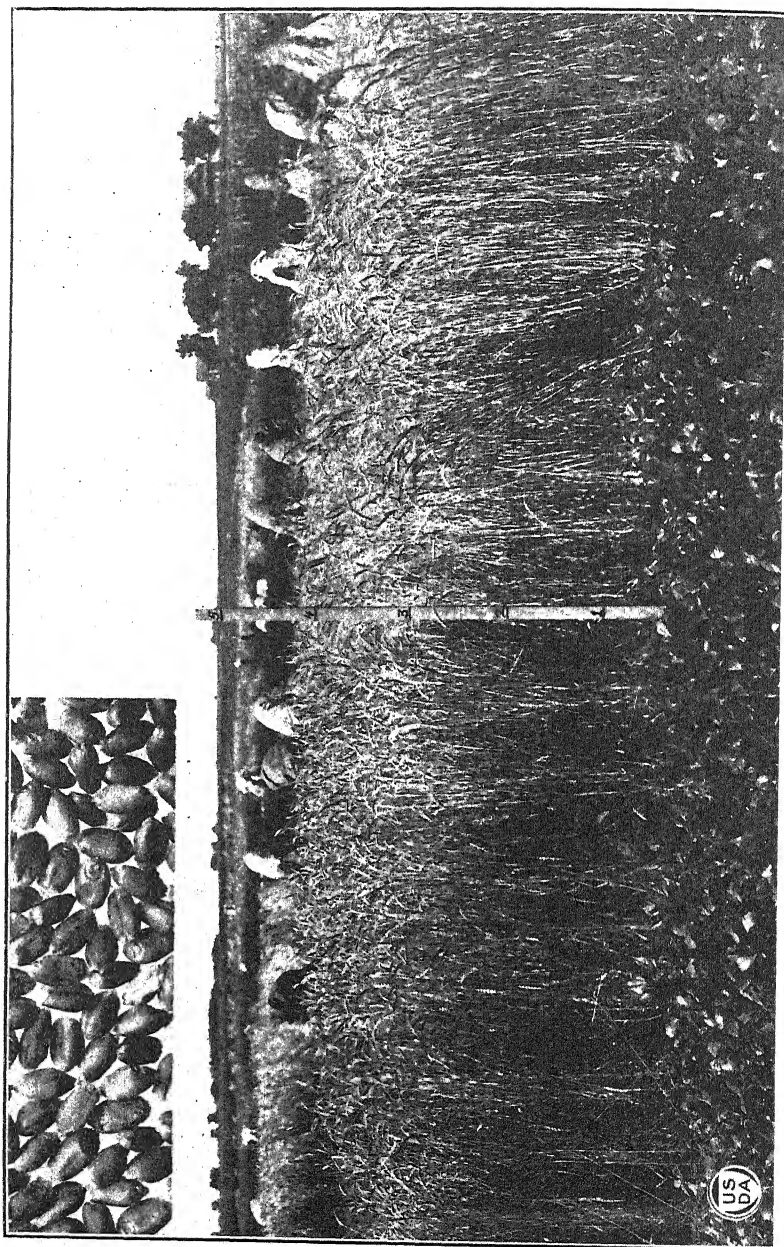


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# PLATE 1

Photograph of plat 8 in the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis on the left and hybrid on the right. This plat had received acid phosphate at the rate of 1,000 pounds per acre in 1916 but none in 1917. It had received sodium nitrate at the rate of 1,000 pounds per acre in 1917. Note the very severe lodging. This is characteristic of the effect of nitrogen on University Farm. Note also the badly shriveled seed from the Marquis. This variety showed 60 per cent of stem rust, and the yield was 15.8 bushels per acre. Compare with Plates 2 and 3.



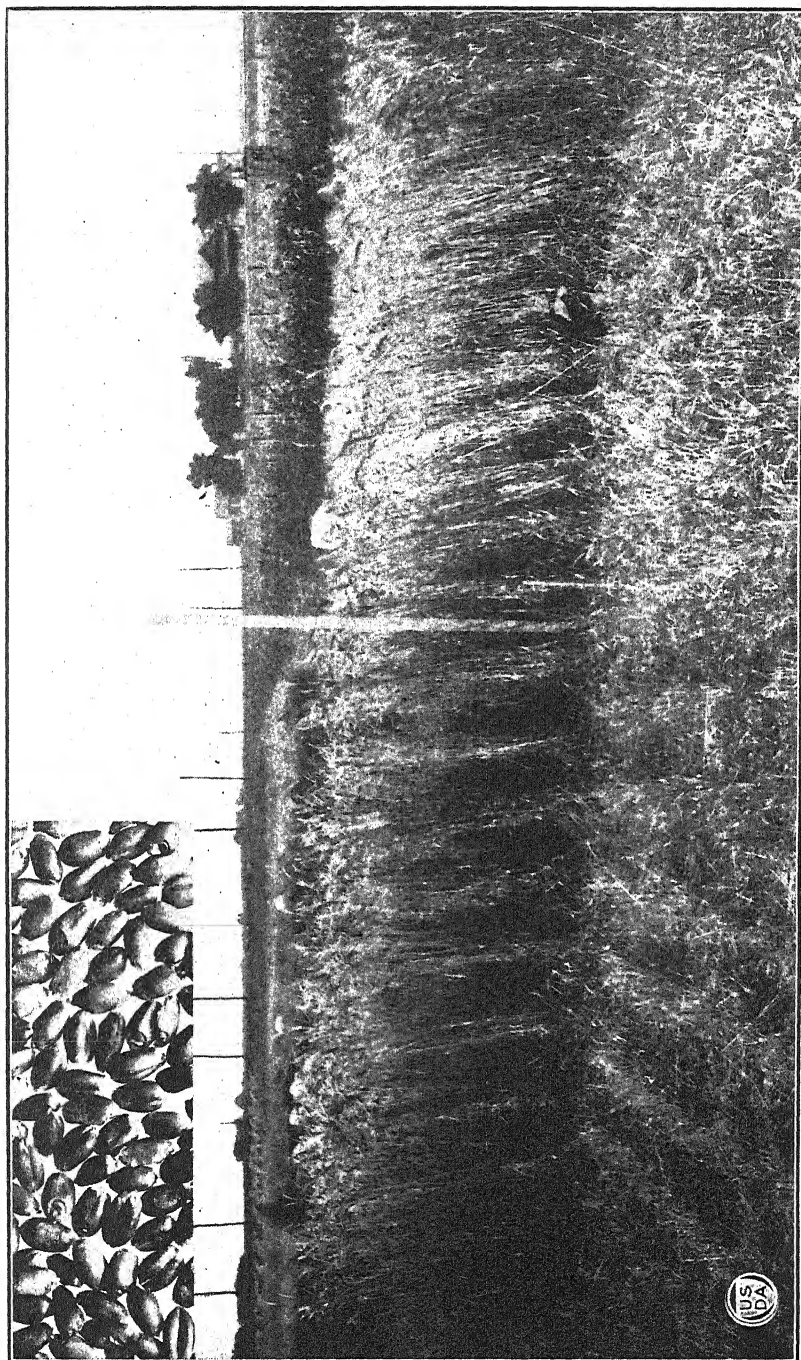


## PLATE 2

Photograph of plat 21 of the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis on the left and hybrid on the right. This plat had received potassium chlorid at the rate of 250 pounds per acre and acid phosphate at the rate of 500 pounds per acre in 1916, but no fertilizer in 1917. Note the uniform stand and freedom from lodging, in sharp contrast with the condition shown in Plate 1. Note also the plump kernels of Marquis compared with the very shriveled kernels shown in Plate 1. The percentage of stem rust was 27 per cent on Marquis and 2 per cent on the hybrid, and the yield of Marquis was 32.6 bushels per acre. The wheat in many of the plats fertilized with acid phosphate and potassium chlorid was like this. The kernels were plump and the yield was satisfactory in spite of the fact that on some of them the rust infection in previous years was from 50 to 85 per cent.

### PLATE 3

Photograph of plat 26 of the commercial fertilizer series in 1917 on University Farm (see Table IX). Marquis wheat on the left and hybrid on the right. This plat received potassium chlorid at the rate of 1,000 pounds per acre in 1916 but no fertilizer in 1917. The rust infection was 32 per cent on Marquis and 7 per cent on the hybrid. The yield on Marquis was 33.2 bushels per acre. Note the plump kernels of Marquis and compare with those shown in Plate 1.







# MORPHOLOGICAL AND PHYSIOLOGICAL STUDIES ON THE RESISTANCE OF WHEAT TO PUCCINIA GRAMINIS TRITICI ERIKSS. AND HENN.<sup>1</sup>

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## INTRODUCTION

Many conflicting statements have been made regarding the nature of resistance of wheat varieties to *Puccinia graminis tritici* Erikss. and Henn. It is clear, also, that there are many different opinions regarding the degree to which this resistance can be changed. For this reason it seemed desirable to investigate the factors which affect the virulence of rust attacks.

There are two conceivable reasons for the resistance of plants to fungous attack—morphological peculiarities and physiological resistance.

There may be mechanical obstacles to the entrance of the germ tubes of the rust fungus into the tissues of the host. While the host cells may be quite susceptible after the germ tubes once have entered, it is quite conceivable that so few may enter that the plant is protected against heavy attack. The number of hairs on the surface of the plant, and the number, size, disposition, and peculiarities of movement of the stomata, conceivably could exert a considerable effect upon the entrance of the germ tubes.

If the pathogene is unable to obtain proper food materials from a certain plant, even after it has entered, the possibility of its parasitizing that plant is precluded, of course. Furthermore, if toxic substances occur in the tissues of the plant, the pathogene will be weakened or killed. It is possible, however, that the cells of certain tissues may be quite susceptible to the attacks of a pathogene but that the number of these cells may be so limited as to make it impossible for the organism to develop extensively. A plant therefore may be physiologically susceptible and morphologically resistant. There appeared to be good evidence from field observations that certain varieties of wheat apparently were resistant to certain biologic forms of *P. graminis* merely because there were not sufficient tissues in the host plant in which the rust parasite could develop. If the plant develops a large amount of woody tissues, it is clear that the area in which rust hyphae can grow is rather limited.

It is well known that the morphology of wheat plants may be affected by temperature, light, moisture, and soil nutrients. Just to what extent environmental factors could affect the development of rust indirectly by affecting the structure of the plant has not been shown. It frequently

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<sup>2</sup> The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for suggesting the problem and for much helpful criticism during its prosecution.

has been observed that varieties which may be susceptible in the seedling stage apparently are resistant in the field. That is, the rust does not develop on them sufficiently to do any damage. The indications are that either the germ tubes fail to enter the host, or, if they do, the mycelium for some reason can not develop extensively. Attention has been focused on this phase of the problem particularly since the discovery of numerous biologic forms of *P. graminis tritici*.

The specific problems which the writer undertook to investigate, therefore, were the following: (1) Are the morphological differences in different varieties of wheat sufficiently great to affect materially the entrance of germ tubes? (2) After the germ tubes have entered, are there structural and physiological peculiarities which account for the inability of certain biologic forms to develop in certain varieties of wheat? (3) Can these structural and physiological characters be altered sufficiently by environmental factors to change the reaction of the host to the rust parasite?

#### HISTORICAL REVIEW

Much of the literature on the subject of rust resistance has been reviewed elsewhere, so only a brief review will be given of those papers which bear definitely on the problems at hand.

✓ The relation of the morphology of the host to rust resistance has been studied by several investigators. Sappin-Trouffy (47)<sup>3</sup> stated that the mycelium of *Puccinia graminis* is entirely localized in the chlorophyll-bearing parenchyma, so that it can grow readily only longitudinally in the stem, being prevented from extensive radial spread by the hemispherical sheath of sclerenchyma fibers. The natural result is the formation of linear pustules. ✓

✓ Cobb (8) thought that the degree of rust resistance of wheat varieties was correlated with the tensile strength of the leaves, the ratio of sclerenchyma to chlorenchyma, the amount of waxy bloom, the number and size of stomata, and the number and length of leaf hairs. ✓ Petermann (40, p. 15-16) suggested that silica increased the strength of cell walls and their resistance to puncture, thus increasing resistance. Farrer (13) was of the opinion that wheat plants with narrow erect leaves and thick epidermis were likely to be resistant. Ward (57), Eriksson and Henning (12), and Biffen (6), on the other hand, concluded that external morphology had little effect on resistance to various rust fungi. ✓

✓ Ward (57) found that *Puccinia glumarum* entered susceptible and resistant hosts equally well, but that the mycelium could not develop normally in the latter. The hyphae soon died in the resistant host, either on account of starvation or poisoning. ✓ Gibson (16) showed that germ tubes of rust fungi easily gained entrance through the stomata of many plants, but that the hyphae could produce haustoria and develop subsequently only in susceptible forms. Marryat (34) in general confirmed the observations of Ward and Gibson and showed further that the cells of wheat varieties resistant to *P. glumarum* were killed by the rust hyphae, which then also died. ✓ Stakman (51), Allen (2), and Newton (37) found that essentially the same thing was true of *P. graminis*. Allen (1) further showed that the size and shape of the guard cells may be a factor in the entrance of the germ tube. ✓

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 408.

Ward (58), Arthur (4), and Stakman (50) emphasized the importance of the effect of the physiologic condition of the host plant on the development of rust. Their general conclusion was that the vigor of the parasite is directly proportional to the vigor of the host. Biffen (6) concluded that the virulence of *P. glumarum* is dependent on the amount of fertilizer used.

It has long been considered that the plane of metabolism of the host profoundly influenced the degree of development of rust. See Raines (42). See also paper of Stakman and Aamodt in the present number of this Journal. The opinion has been prevalent that heavy applications of nitrogen increased the susceptibility of cereals to rusts. See Sorauer (48), Hiltner (23), Comes (10), Spinks (49), Little (29), Bolley (7), Anderson (3), Remer (43), Freeman (14), Cobb (8), Stakman and Aamodt (53). It has also been thought that potassium and phosphates rendered them more resistant. See Montemartini (35), Müller and Molz (36), Sorauer (48), Hiltner (23), Comes (10), Spinks (49), Remer (43). On the other hand, Pantanelli (39) found that excess phosphate in relation to nitrogen increased resistance only when it checked the growth of the host plants. Stakman (52) states that large amounts of nitrogenous fertilizers, particularly on soils which do not need them badly, will permit greater rust damage. Plants so fertilized have a weak straw which crinkles badly when rust attacks it. The development of a stiff straw is desirable, and plants fertilized with potassium or phosphate fertilizers usually yield better in bad rust years than those heavily fertilized with nitrogen.

As to the influence of certain physicochemical properties of the sap of the host plant upon rust development, Comes (10) concludes that resistance of wheat to *P. glumarum* is increased by superphosphate fertilizer but is weakened by nitrogenous fertilizer, and suggests that this increased resistance might be due to an increase in the acidity of the sap. He states that the sap of "Rieti" wheat is more acid than that of any other Italian wheat. This variety is particularly resistant to rust. Kirchner (27) and Henning (22) also call attention to the importance of high acidity of the cell sap in resistance to rust. Gortner (17) reports that excess fertilization with sodium nitrate decreased the hydrogen-ion concentration in Haynes Bluestem wheat. Grimaldi (19) and Hurd (24) question the importance of the acidity of the cell sap in resistance of wheat to rust, pointing out that there is no positive correlation between the two. The latter shows that environmental conditions may cause greater differences in the acidity of the cell sap of a single variety than usually occur between different varieties grown under the same conditions. Pantanelli (39) states that the organs of the wheat plant most susceptible to rust are those richest in sugars, acids, and in soluble compounds of phosphorus and nitrogen.

Mains (32) and others have shown that the carbon metabolism of the host is important in the development of rust. Kirchner (27) and Henning (22) give tables of the sugar content of the saps of resistant and susceptible varieties. These tables indicate that the concentration of glycose or reducing sugars is higher in the more susceptible varieties.

#### EXPERIMENTAL DATA

Studies have been made on the entrance of germ tubes of *Puccinia graminis* into the host, the morphology of the wheat plant as related to rust resistance, the relation of nutrient salts to the development of stem

To determine the relative number of stomata on the leaf, seedlings of wheat varieties were grown in the greenhouse. When the seedlings were 10 days old, the first leaf of several plants of each variety was cut off near the base and placed in aceto-alcohol until clear. The entire leaf was then mounted in aceto-alcohol and observed through strong transmitted light. Counts were made both on the midrib and at the outside of the leaf, 1 inch and 2 inches from the tip.

TABLE III.—Average number of stomata in 10 fields, each containing 2.138 sq. mm., from both surfaces on different parts of the leaves of five wheat varieties

Variety.	On midrib.		Edge of blade		Average for leaf.	Average both surfaces.
	Distance from tip.		Distance from tip.			
	1 inch.	2 inches.	1 inch.	2 inches.		
Khapli, C. I. 4013:						
Lower surface.....	56	57	52	49	54	} 66
Upper surface.....	69	80	78	87	78	
Kanred, C. I. 5146:						
Lower surface.....	37	47	42	45	43	} 64
Upper surface.....	70	78	71	81	75	
Marquis, C. I. 3641:						
Lower surface.....	52	34	45	28	40	} 61
Upper surface.....	79	78	92	84	83	
Little Club, C. I. 4066:						
Lower surface.....	44	50	44	38	44	} 61
Upper surface.....	70	81	73	89	79	
Kota, C. I. 5878:						
Lower surface.....	36	35	32	33	34	} 45
Upper surface.....	56	55	59	56	56	

TABLE IV.—Size of stomatal apertures on seedling leaves of different wheat varieties in the greenhouse, at different times in a winter day with bright sunlight from 9.45 a. m. to 4.35 p. m., at University Farm, St. Paul, Minn.

Variety and data on stomatal openings.	Time of day.				
	9.15	11.15	1.15	3.15	5.15
Little Club, C. I. 4066:					
Aperture in micra...	32.1 x 0.5	34.3 x 4.35	40.3 x 7.4	38.9 x 7.8	Closed.
Percentage open...	1	32	100	100	0
Khapli, C. I. 4013:					
Aperture in micra...	22.6 x 0.5	22.6 x 0.5	22.6 x 0.5	24.2 x 0.5	22.6 x 0.5
Percentage open....	1	60	75	20	5
Kanred, C. I. 5146:					
Aperture in micra...	34.2 x 0.5	35.9 x 5.1	39.4 x 6.3	40.5 x 5.0	Closed.
Percentage open....	60	95	95	95	0
Mindum, C. I. 5296:					
Aperture in micra...	23.5 x 0.5	28.1 x 1.0	31.3 x 0.9	26.1 x 0.7	31.3 x 0.5
Percentage open....	40	80	10	10	5
Marquis, C. I. 3641:					
Aperture in micra...	Closed.	33.0 x 1.3	33.0 x 1.3	Closed.	Closed.
Percentage open....	0	1	1	0	0
Kota, C. I. 5878:					
Aperture in micra...	35.0 x 0.5	36.9 x 2.1	35.1 x 2.9	35.1 x 3.1	Closed.
Percentage open....	20	20	70	1	0

There is no great difference in the number of stomata on the different varieties: Kota always has the least, and Khapli consistently the most. (Table III.) The difference in the number of stomata on these varieties seems unrelated to the degree of resistance to stem rust in the greenhouse. Khapli, the most resistant wheat used for determining biologic forms of rust at University Farm, has the largest number of stomata. What appears to be more pertinent to the problem is the frequency and the extent to which the stomata open.

#### SIZE AND MOVEMENT OF STOMATA

Allen (1) suggested that the inability of many germ tubes to gain entrance into Kanred wheat might be due to the size and shape of the stomatal aperture.

The writer compared the size of the aperture of stomata of five wheat varieties. The method employed by Lloyd (30) was used. Epidermis strips were placed immediately into absolute alcohol, and then studied under the microscope. Check observations were made by fastening growing leaves to the substage of the microscope and observing the stomata direct. Measurements made in this manner agreed entirely with those from epidermis strips placed in alcohol.

It is obvious from these observations that the stomatal slits of Kanred wheat are open to a much greater extent than would be expected from Allen's preliminary report. These data are shown in Table IV.

Allen (1) found that only about 10 per cent of the germ tubes of a form from which Kanred is immune entered this variety, while about 30 per cent of the germ tubes of a form to which Kanred is susceptible entered. The following explanation was offered: "The presence of the appressorium might act as a stimulus by mere contact, by altering the gaseous exchange through the stoma or disturbing the moisture relations, by exerting a possible toxic influence upon the guard cells, or by its presence shutting off some of the light from the guard cells. It is at least conceivable that the guard cells might be sensitive to the appressorium and remain closed, thus excluding the fungus." She also observed that in Baart, a variety very susceptible to most biologic forms of *P. graminis tritici*, only 67 per cent of the germ tubes which formed appressoria entered.

Newton (37) has studied the behavior on Kanred of the germ tubes of a biologic form which fails to produce pustules or flecks on this wheat variety, and concludes that approximately 30 per cent of the germ tubes which form appressoria enter. This difference in the observations of different investigators strongly suggests that conditions during the incubation period influence the number of entries which the fungus is able to make. It seems probable, therefore, that the conditions which influence the opening of the stomata, both before and after appressoria are formed, may have considerable effect on entrance.

Loftfield (31) points out that the stomata of cereals rarely are all open to their maximum at the same time. Even under the most favorable field conditions all the stomata are open only for one or two hours each day, the tendency in cereals being to operate with many closed stomata. He further observed that on many days the stomata of wheat plants do not open at all.

On the two days prior to the date of observations, careful examination showed that the stomata of the six wheat varieties used in the experiment

presented in Table IV failed to open. These days were very dark, there being no sunlight whatever. It seems that stomatal movement not only varies with different varieties of wheat, but varieties themselves evidently do not respond to a variable in exactly the same way.

Experiments therefore were made to ascertain whether the stomatal behavior in any particular variety of wheat is an important factor in determining the number of stem-rust infections which result from a given number of spores germinating on the leaf surface.

#### RELATION OF FORMATION OF APPRESSORIA TO SUBSEQUENT INFECTION

Seedlings of five wheat varieties which behave quite differently toward several biologic forms of *P. graminis* were heavily inoculated with viable urediniospores and placed in humidity chambers for 48 hours. The plants then were placed on the greenhouse bench for 48 hours. A number of leaves then were taken at random from each variety and cleared several days in aceto-alcohol. The remaining leaves were left in order to ascertain the number of uredinia which would develop. The cleared leaves then were submerged one minute in Pianezze stain, and washed in water to remove the excess stain. The leaf tissue which had been cleared by the aceto-alcohol did not take the stain, but the germinating spores, germ tubes, and appressoria on the leaf surface were stained purple red. The entire leaf was then mounted, and the germ tubes on its surface were observed in direct light. The results are given in Table V.

TABLE V.—Relation between number of appressoria of *Puccinia graminis* produced on the wheat leaf and subsequent infection

Wheat variety.	Percentage of urediniospores germinating on leaf.	Average number of appressoria per leaf.	Number inoculated plants infected. <sup>a</sup>	Average number of uredinia per leaf.
INOCULATIONS WITH BIOLOGIC FORM XVIII				
Kanred, C. I. 5146.....	Abundant; approximately 50 per cent.	17	7	3.2
Little Club, C. I. 4066.....		20	10	2.8
Kota, C. I. 5878.....		15	9	2.1
Marquis, C. I. 3641.....		19	8	2.7
Khapli, C. I. 4013.....		20	9	4.6
INOCULATIONS WITH BIOLOGIC FORM XXVII				
Kanred, C. I. 5146.....	Abundant; approximately 50 per cent.	32	0	0
Little Club, C. I. 4066.....		44	8	2.9
Kota, C. I. 5878.....		17	0	0
Marquis, C. I. 3641.....		27	7	2.3
Khapli, C. I. 4013.....		23	9	4.2

<sup>a</sup> The denominator indicates the number of leaves artificially inoculated, and the numerator shows the number which became infected.

The degree of infection produced by biologic Forms XVIII and XXVII on the wheat varieties given in Table V is summarized below. These data are based on Table IV, Technical Bulletin No. 8, Minnesota Agricultural Experiment Station.

Wheat variety.	Degree of infection.	
	Biologic Form XVIII.	Biologic Form XXVII.
Kanred, C. I. 5146.....	Very heavy.....	No infection.
Little Club, C. I. 4066.....	do.....	Very heavy.
Kota, C. I. 5878.....	do.....	No infection.
Marquis, C. I. 3641.....	do.....	Weak.
Khaphi, C. I. 4013.....	Very weak.....	Do.

On some leaves observed in the foregoing study, as many as 60 appressoria were seen, and where the spore germination was profuse there never were fewer than 15. As relatively few uredinia developed, there appeared to be but little correlation between the number of appressoria formed and the number of subsequent infections. For example, it frequently has been observed that even on the completely susceptible Little Club, numerous appressoria may be observed on leaves which fail to become infected. It seems improbable that the germ tubes entered, because antagonism between the host cells and the parasite, which conceivably could account for lack of infection, never has been observed in this variety.

It is probable that the stimuli for the formation of appressoria and for subsequent entrance of the germ tube are entirely different. The formation of appressoria may be due to thigmotropism, because they often are formed in depressions between epidermal cells and in other places where they come in contact with some object. Ward (59), Robinson (44), Fromme (15), and Mains (32) noted that the germ tubes of certain rust fungi tended to be negatively heliotropic; while Balls (5) suggested that a hydrotropic response might account for entrance. The germ tubes might grow toward the stomatal opening, either on account of a negatively heliotropic or a positively hydrotropic response, or both; but the tubes probably can not enter if the stomata are closed tightly.

According to Allen (1), only 67 per cent of the germ tubes which form appressoria enter the very susceptible host, Little Club. If only two-thirds of the germ tubes forming appressoria enter a very susceptible host under favorable conditions, we are not justified in emphasizing the relative number of germ tubes which enter from appressoria formed on resistant hosts. Before the results of different experiments can be compared, it will be necessary to control all the factors which influence the movement of stomata during the incubation period.

It is quite possible that neither the number nor the size of the stomata greatly influences the entrance of the germ tubes. It is probable that the degree and duration of opening are more important. If the stomata are fairly wide open for some time the germ tubes may enter easily, but if they remain closed, there is no evidence that the tubes can force their way into the substomatal chamber. On the other hand, there is abundant evidence to show that even on the most susceptible varieties spores



may produce abundant germ tubes which form appressoria over the stomata and yet do not enter. In these cases it is possible that the stomata may not have opened at all.

It appears probable that entrance in itself, so far as greenhouse studies are concerned, is a factor in the resistance of most wheat varieties only when the stomatal movement is such as to effect complete closure of the aperture. The very fact that many biologic forms regularly enter Kanred seedling leaves under greenhouse conditions is sufficient evidence that the failure of some forms to produce infection can not be due to the small size of the aperture. It seems more probable that the forms which do not enter may have a specific physiological effect upon the guard cells, which respond by complete closure, or that when entrance is effected the germ tubes are destroyed before they can produce any easily visible effect upon the host.

#### MORPHOLOGY OF THE WHEAT STEM AS RELATED TO RUST RESISTANCE

During recent years the idea has been prevalent that rust resistance was not due to morphology of the host. But it never has actually been demonstrated that the morphology of the wheat stem bears no relation to the development of *Puccinia graminis*. On the other hand, there are certain suggestions in the early literature that the internal structure of the stem may determine in some measure the extent of spread and development of the fungus. This is particularly evident in the plates and figures by Sappin-Trouffy (47) and Eriksson and Henning (12).

*P. graminis* causes the greatest injury when it attacks the portion of the stem or peduncle immediately below the rachis, known as the neck. It is evident from field observations that the necks of all varieties are not uniformly attacked by stem rust. Plate 1, F and G, shows two varieties of wheat, one quite susceptible and the other generally resistant to stem rust, in the field plots at University Farm, St. Paul.

The variety shown in Plate 1, F, is Kota, C. I. 5878, which is generally considered resistant in the field. It is attacked by stem rust, however, and normal linear uredinia are produced. These may be as much as a centimeter in length. Usually the uredinia are separate and quite distinct to the eye. Even when the infections are numerous, it is not difficult to make out the characteristically linear individual uredinia. The spores produced are quite normal.

Plate 1, G, shows the heads and necks of Little Club, C. I. 4066. This variety is always very severely rusted at University Farm, St. Paul. It sometimes is so severely injured by stem rust that, when heads are produced, only very small shriveled kernels are formed. The uredinia just below the rachis are very large and confluent. The epidermis breaks off as large scales, exposing masses of spores in a single large uredinium beneath.

#### STRUCTURE OF THE STEM

In order to study the structure of the stem, several wheat varieties were grown in adjacent rows. When the heads were in the soft-dough stage, stems were killed and cleared in aceto-alcohol. Sections 10 $\mu$  thick were then cut in pith by means of a sliding razor. Hydrochloric acid-orcin, hydrochloric acid-phloroglucin, and analin-sulphate were used to differentiate the cellulose and pentosan structures. For permanent mounts for photomicrographs, sections were stained with Planeze stain,



passed through acid alcohol, cleared in carbol-turpentine, passed through xylol and then mounted in balsam. Light staining with iodine clearly brought out the chlorophyll-bearing areas of the stem.

The wheat stem consists of a hollow cylinder, except in solid-stemmed varieties. (Pl. 2, A and B.) The epidermis, of course, consists of a single layer of cells, the inner walls of which are sometimes lignified. The number of stomata per unit area of surface differs in different varieties. Just beneath the epidermis is the chlorenchymatous collenchyma. This sometimes extends in an almost continuous band around the entire stem, although it usually is interrupted by strands of sclerenchyma. The collenchyma cells then are aggregated into isolated bundles, the size and number of which vary considerably in different varieties. The vascular bundles are arranged in a more or less definite ring in the thin-walled parenchymatous tissue of the fundamental ground tissue. Toward the inside is the pith. The bundles may be arranged in one or more rings; usually there are about 15 large bundles and a variable number of smaller bundles outside of the larger ones. A more or less continuous sheath of sclerenchymatous fibers extends between the different bundles and outside of them. Strands from this sheath often extend from the bundles through the collenchyma to the epidermis. It is particularly important to remember that the only chlorenchymatous tissue in the stem is the collenchyma. These extensions of the sclerenchymatous tissue interrupt the continuity of the collenchyma and break it up into bundles, the size of which depends upon the amount of sclerenchymatous tissue which develops.

This same general structure was shown by Sappin-Trouffy (47, p. 103) for oat stems and is figured for the sheath of wheat by Eriksson and Henning (12, pl. 4, fig. 40 b).

Photographs of sections of the upper peduncle or neck of four varieties of wheat—Kota, C. I. 5878; Little Club, C. I. 4066; Marquis, C. I. 3641; and Sonem emmer, C. I. 4402—are shown in Plate 2, C, D, E, and F. It is evident that the relative proportion of sclerenchyma to collenchyma is not the same in each variety. In Kota the sclerenchyma divides the collenchyma into distinct areas, while in Little Club the sclerenchyma is much less conspicuous and the collenchyma is practically continuous. In the Marquis stem there are somewhat more sclerenchyma fibers than in the Little Club stem and there also is a greater tendency for union of the collenchyma areas than in the stem of Kota. In the stem of Sonem emmer a large amount of sclerenchyma always is developed.

The morphological difference which may be expected in stems of different wheat varieties is illustrated in figure 1. The illustrations are actual tracings of permanently mounted sections prepared in the course of these experiments. In order to secure these tracings, the slides bearing the sections were placed on the stage of the microscope attachment of a projection lantern. The sections were then projected on white cardboard, placed 6 feet from the lantern. This greatly enlarged the sections, so that the details of structure could easily be traced with a pencil. The epidermis and fibrovascular bundles, together with all sclerenchyma, then were filled in with India ink, following which tracings of the different varieties were placed on a single large card and photographed. The interesting fact is the marked difference in the extent and distribution of the collenchyma areas in the different varieties. Figure 1, A, is made from a tracing of a section of Sonem emmer, C. I. 4402. In three years' observation this variety has shown less infection from both *P. tritici*

and *P. graminis* than any other wheat grown at University Farm, St. Paul. The collenchyma areas are extremely small and make up but little of the stem structure. The sclerenchyma areas are decidedly predominant in this variety and make up the major portion of the stem proper. The unevenness of the surface made by the bulging out of the

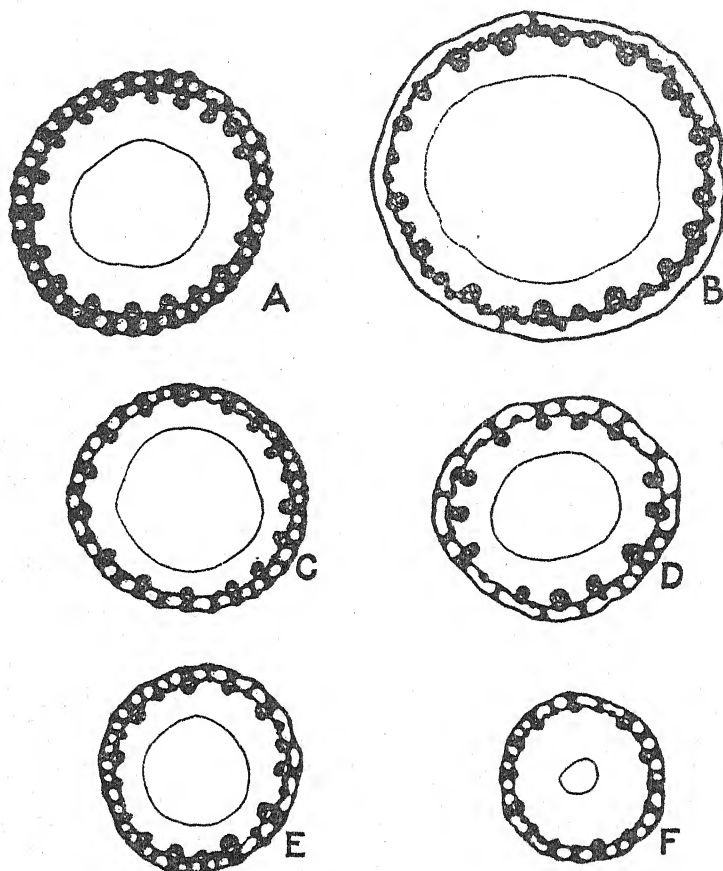


FIG. 1.—Tracings of transverse sections of the upper peduncle or neck of different wheat varieties. The sclerenchymatous tissues are represented by the black areas. Note the practically continuous band of collenchyma just under the epidermis in B; note also the relatively large collenchyma bundles in D. (X 25.)

- A.—Sonem emmer, C. I. 4402.
- B.—Little Club, C. I. 4066.
- C.—Vernal (White Spring) emmer, C. I. 3686.
- D.—Marquis, C. I. 3641.
- E.—Kota, C. I. 5878.
- F.—Einkorn, C. I. 2433.

sclerenchyma between the chlorophyll-bearing areas is very prominent. This condition is characteristic of all the emmer varieties studied, although the unevenness is particularly evident on Sonem emmer.

Figure 1, B, shows a tracing of a section of Little Club, C. I. 4066. This variety as infected by rust in the field, is shown in Plate 1, E.

Sections of its stem also are shown in Plate 2, D. In marked contrast to figure 1, A, the chlorophyll-bearing area is quite extensive and continuous. The sclerenchyma is confined to the regions between the fibrovascular bundles. The surface of the stem is smooth and even. Figure 1, C, is of Vernal (White Spring) emmer, C. I. 3686. This variety is similar to Sonem emmer in the extensive development of sclerenchymatous tissues. A tracing of a section of the stem of Marquis, C. I. 3641, is shown in figure 1, D. In this variety the collenchyma areas are smaller than similar tissues in Little Club, but, nevertheless, they are considerably larger than the collenchyma areas of the emmer varieties. Figure 1, E, is a tracing of Kota, C. I. 5878. A photograph of the variety is shown in Plate 1, F. Figure 1, F, shows a tracing of Einkorn, C. I. 2433, a variety of wheat possessing a stem of small diameter, compared with that of other varieties. All of the tracings shown in figure 1 have been traced on the same scale and show relative size.

It must be kept in mind that the diagrams shown illustrate the tendency of a variety to produce a certain type of structure and that certain fluctuations are to be expected in individuals. Even Little Club, when sectioned some distance from the rachis, shows the chlorophyll areas, beginning to be divided by sclerenchyma, but never to the extent shown in other varieties. Plate 1, F, shows this fact in that the pustules at a distance from the rachis are taking on a more elongated appearance. On the other hand, out of hundreds of sections made, never has a section of emmer shown any tendency of the collenchyma areas to be confluent. Considering the established fact that the development of the mycelium of *P. graminis* is practically limited to the collenchyma of the stem, it is not difficult to see that the different varieties studied will necessarily not show the same amount of stem-rust injury when subjected to a rust epidemic.

#### STEM STRUCTURE AS RELATED TO RUST DEVELOPMENT

Sappin-Trouffy (47, p. 93) states that the action of *P. graminis* on oat stems is quite restricted, as the mycelium is unable to extend except longitudinally, because it is bounded at the sides by a hemispherical sheath of sclerenchyma fiber. The same is true for the action of *P. graminis* on wheat stems. On the Kota stem the uredinia are linear each one being confined to a single collenchyma bundle. (Pl. 2, G.) In the neck of Little Club the mycelium is not limited by sclerenchyma strands but is free to extend laterally and therefore produces very large pustules. (Pl. 2, H.) Comparing these photographs with Plate 1, F and G, the importance of the sclerenchyma in relation to the size of the pustule is clear.

If the opportunities for infection are exceptionally favorable and a large number of individual infections result, even the varieties with extensive sclerenchyma may become severely rusted. Under such conditions more susceptible varieties, such as Little Club or Marquis would be seriously injured. It is quite possible, on the other hand, that when only a few infections take place, these latter varieties may be severely attacked, while the emmer varieties may escape comparatively unharmed. The structure of the stem affects only the extent of the spread of the organism and its subsequent rupture of the epidermis. Resistance to stem rust must be considered as being due fundamentally to a complex physiological relationship. The results obtained by Stakman and Levine (54) show the difficulty in explaining actual resistance of wheat varieties

on morphologic grounds alone. Actual resistance is in its finality a matter of the individual cell. We may picture the collenchyma cells of the neck of Little Club wheat as so many easily rotted fruits in a large container where the fungus may spread quickly throughout the entire mass, infecting them all. The collenchyma cells in the stem of such a variety as Kota or Vernal (White Spring) emmer, when attacked by a biologic form to which it is susceptible, would be like the same fruit packed in small baskets, so that when one container was infected the fungus could not pass to the next. Thus a large part of the fruit would remain uninjured. On the other hand, if one conceives two kinds of fruit, one resistant and the other susceptible, both of them packed alike, the amount of actual injury sustained is placed on an entirely different basis. Of the two bases, the latter is a more fundamental type of resistance, and it is the type emphasized by Stakman and Levine in identifying biologic forms. Acme, C. I. 5284, a wheat variety reported by Stakman and Levine to be highly resistant to only 1 biologic form in 37, suffers very little from rust attacks in the field at University Farm, St. Paul. A section of the neck of this variety is shown in Plate 2, I. It has a strong tendency to produce sclerenchyma fibers between the epidermis and the collenchyma, a characteristic not observed in any other variety studied. The amount of sclerenchyma shown in Plate 2, I, is unusually large for Acme.

The diameter of the stem may have some effect on the amount of injury caused by rust. Other factors being equal, the diameter of the stem alone would tend to determine the resistance to rupture, etc. The smaller diameter gives the greater surface curvature with the greater resistance to rupture. That this resistance to rupture may be of some significance is shown by the fact that the mycelium may develop within the collenchyma areas of certain small-stemmed varieties with abundant sclerenchyma, and yet produce no uredinia. This condition is quite common in the emmers. Within a given variety, any factors which would increase the size of the stem or the proportion of collenchyma to the sclerenchyma would also tend to increase the actual injury resulting from a rust attack. The many statements by investigators concerning the heavier rust infections due to manuring with nitrogenous fertilizers may be in some way partly connected with this fact.

#### RELATION OF NUTRIENT SALTS TO THE DEVELOPMENT OF STEM RUST

A series of sand cultures was set up for the purpose of studying the relation of certain nutrient salts to the morphology and physiology of the wheat plant, and the possible indirect influence of these salts on the development of stem rust.

#### EXPERIMENTAL PROCEDURE

A clean, white quartz sand was secured from the division of soils of the Minnesota Agricultural Experiment Station. This was best Ottawa quartz and contained so little nutrient material as to be unable to support growth. All the salts used in making up the nutrient solutions were chemically pure but were not recrystallized.

In setting up this series of cultures two liters of sand were placed in each crock, to which was added 1,900 cc. of distilled water containing the entire amount of the salts used. A constant water table was maintained by adding distilled water. A water gauge was inserted in the hole at the bottom of the crock and paraffined in to insure a ready means of observing the water table for each pot. In addition to the water gauge from the bottom of the crock, an inverted thistle tube was placed in the center, so that the bell of the tube, covered with cheesecloth, was 2 inches from the bottom. This furnished good aeration and permitted excellent root development.

Two series were set up on different dates, January 29, 1922, and June 14, 1922. Haynes Bluestem wheat, C. I. 2874, from the same seed lot, was sown in each series. Tables VI and VII give the salt combinations used.

TABLE VI.—Salts used in series of sand cultures started January 29, 1922, to determine the relation of nutrient salts to development of stem rust

Series No.	Salt.	Grams per liter.	Gram molecules per liter.
101.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	0. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
102.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
	$\text{KNO}_3$ .....	1. 729	. 0171
103.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
	$\text{MgHPO}_4$ .....	2. 982	. 0171
104.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
	$\text{K}_2\text{SO}_4$ .....	2. 980	. 0171
105.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
	$\text{NaNO}_3$ .....	1. 454	. 0171
106.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030
	$\text{CaHPO}_4$ .....	2. 943	. 0171
107.....	$\text{Ca}(\text{NO}_3)_2$ .....	1. 279	. 0078
	$\text{KH}_2\text{PO}_4$ .....	1. 471	. 0108
	$\text{MgSO}_4$ .....	0. 361	. 0030

TABLE VII.—Salt combinations used in series of sand cultures started on June 14, 1922, to determine the relation of nutrient salts to development of stem rust

Series No.	Salt.	Grams per liter.	Gram molecules per liter.
121.....	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	0. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
	MgSO <sub>4</sub> .....	0. 361	. 0030
	FeSO <sub>4</sub> .....	0. 020	. 0001
122.....	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
	MgSO <sub>4</sub> .....	0. 361	. 0030
	FeSO <sub>4</sub> .....	0. 020	. 0001
123.....	K <sub>2</sub> HPO <sub>4</sub> .....	1. 882	. 0136
	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
	MgSO <sub>4</sub> .....	0. 361	. 0030
124.....	FeSO <sub>4</sub> .....	0. 020	. 0001
	KNO <sub>3</sub> .....	0. 864	. 0015
	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
125.....	MgSO <sub>4</sub> .....	0. 361	. 0030
	FeSO <sub>4</sub> .....	0. 020	. 0001
	CaHPO <sub>4</sub> .....	2. 943	. 0171
	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
126.....	CaHPO <sub>4</sub> .....	0. 294	. 0017
	MgSO <sub>4</sub> .....	0. 361	. 0030
	KNO <sub>3</sub> .....	1. 729	. 0171
	FeSO <sub>4</sub> .....	0. 020	. 0001
127.....	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
	MgSO <sub>4</sub> .....	0. 361	. 0030
	FeSO <sub>4</sub> .....	0. 300	. 0007
128.....	Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1. 279	. 0078
	KH <sub>2</sub> PO <sub>4</sub> .....	1. 471	. 0108
	MgSO <sub>4</sub> .....	0. 361	. 0030
	AlCl <sub>3</sub> .....	1. 895	. 0171

When the seedlings in the series of January 29 were two months old, in place of the regular watering with distilled water, a quantity of the particular salt being tested for each series was added to each crock in the series as follows:

Series No.	Salt added Apr. 1.
101.....	250 cc. distilled water, no salt.
102.....	250 cc. distilled water, containing 0.432 gm. KNO <sub>3</sub> .
103.....	250 cc. distilled water, containing 0.745 gm. MgHPO <sub>4</sub> .
104.....	250 cc. distilled water, containing 0.745 gm. K <sub>2</sub> SO <sub>4</sub> .
105.....	250 cc. distilled water, containing 0.363 gm. NaNO <sub>3</sub> .
106.....	250 cc. distilled water, containing 0.736 gm. CaHPO <sub>4</sub> .

The plants of this series were then inoculated on April 1, 1922, with urediniospores of biologic Form XXXIII. This form, as shown by Stakman and Levine (54), produces a type "2" infection on Haynes Bluestem. It was hoped that a form which produced a type 2 infection would be more sensitive to changes of the host than a form to which the host is very susceptible or very resistant. The different pots of the same salt combinations were not placed together in the incubation chambers but were scattered at random so as to prevent any two pots with the same salt combination from being together. The plants were so heavily inoculated by dusting with urediniospores that the spore mass could be seen easily on the leaves. The cloth chambers were then covered with wet cheesecloth, an atomizer was inserted through slits along the sides near the top and a fine mist spray was forced into the chamber. The air remained saturated throughout the 48-hour incubation period, following which the plants were placed on the greenhouse bench for 14 days. No observations were made on the stomatal movements, although this would have been desirable.

The series of June 14 was exactly like the previous one except that no additional salt solution was added at the time of inoculation. The plants were inoculated on July 19, when about 5 weeks old. They, therefore, were younger than those of the first series, but they were fully as well developed, owing to the difference in available sunlight.

#### RESULTS OF INOCULATION WITH BIOLOGIC FORM XXXIII

The degree of rust infection on the seedlings is given in Tables VIII and IX.

TABLE VIII.—*The effect of different nutrients on the growth of Haynes Bluestem wheat plants in sand cultures started January 29, 1922, and on the development of stem rust caused by biologic Form XXXIII used as inoculum on April 1, 1922*<sup>a</sup>

Series No.	Changes in basic nutrient solution.	Percentage of plants infected.	Severity of infection.	Character of growth.
101	None (control)....	None.....	.....	Good.
102	KNO <sub>3</sub> added.....	85.....	Medium...	Excessive tillering.
103	MgHPO <sub>4</sub> added..	None.....	.....	Medium.
104	K <sub>2</sub> SO <sub>4</sub> added.....	1 uredinium on 1 plant.	Medium...	Do.
105	NaNO <sub>3</sub> added ....	70.....	...do.....	Fair; much tillering.
106	CaHPO <sub>4</sub> added...	None.....	.....	Medium.

<sup>a</sup> These observations were made by Dr. E. C. Stakman and M. N. Levine.

TABLE IX.—*The effect of different nutrients in sand cultures started June 14, 1922, on the growth of Haynes Bluestem wheat plants and on the development of stem rust caused by biologic Form XXXIII, used as inoculum on July 19, 1922*

Series No.	Changes in basic nutrient solution.	Percentage of plants infected.	Severity of infection.	Character of growth.
121	None (control) .....	60	Medium ...	Good.
122	Phosphate increased by adding $K_2HPO_4$ .	20	...do. ....	Medium.
123	Nitrate increased by adding $KNO_3$ ..	80	Severe....	Excessive tillering.
124	Nitrate further increased by adding twice as much $KNO_3$ as in 123.	90	...do. ....	Do.
125	Nitrate decreased to one-tenth; phosphate increased by adding $CaHPO_4$ .	0	No infection.	Fair; leaves narrow; sandy.
126	Phosphate decreased to one-fifth, using $CaHPO_4$ ; nitrate increased by adding $KNO_3$ .	20	Medium ...	Poor.
127	$FeSO_4$ increased by adding 15 times as much.	50	...do. ....	Good.
128	$FeSO_4$ omitted; $AlCl_3$ added. ....	(a)	.....	Very poor; plants killed by root-rotting fungi.

<sup>a</sup> Not inoculated.

It will be seen from Table VIII that there was no rust on any plants except those grown in sand to which considerable nitrogen had been added. A single exception was one uredinium which developed on a plant in the potassium sulphate series. Table IX shows that in the second series the rust developed more abundantly on the plants fertilized with a large amount of nitrogen. However, in this series the rust failed to develop only on plants receiving a very poor balance of nitrogen and phosphorus.

The plants in the pots receiving excess nitrate tillered more than those in the other pots and developed leaves of a different texture. The lower epidermis was removed from a leaf of a plant receiving an excess of potassium nitrate and compared with that of a leaf from a plant in soil deficient in nitrate. (See Pl. 9.) The epidermis from the plants supplied with abundant nitrogen is smooth, with few hairs and but little bloom, while on plants with a poor balance, or a lack of nitrogen, the leaves are rough to the touch, more hairs are present, the bloom can readily be seen, and there are fewer stomata.

The relation of nutrient salts to the type of cell wall and general character of the epidermis has been briefly mentioned in the historical review. Petermann (40, p. 15-16) states that the cell walls are much stronger in wheat plants on a low plane of nutrition, while manures produced rusted plants, owing to the fact that the haustoria could penetrate more easily. Palladin (38) asserts that cereals grown in soils deficient in silicic acid often rust so severely that it is difficult to prevent their complete destruction. Russell (46) states that nitrogenous manures produce plants with thin walls, easily attacked by fungous hyphae. Comes (9) also maintains that organic fertilizers increase the tenderness of the plant and favor fungous attack.



Bolley (7), Farrer (13), and Cobb (8) state that wheat varieties with stiff, upright growth and narrow leaves are less susceptible to *P. tritici* than are those with broader leaves and less erect habit of growth. Here, again, the germ tubes may have been prevented from entering on account of the failure of the stomata to open. The morphological characters pointed out by Cobb and others can be produced in any variety when it is grown under extreme conditions of soil fertility.

Harter (20) found that wheat plants grown in soils to which had been added from 0.7 to 1.4 per cent sodium chlorid developed a heavy bloom on the leaf surface, thickened cuticle, and epidermal cells of reduced size. This condition may not have resulted from any physiologic action of the salt itself within the plant tissues. These xerophytic characters, together with decreased transpiration, probably are due to the influence of the salt on the entrance of other soil nutrients; and, consequently, only a very poorly balanced nutrition is available to the plants.

It appears from Table VIII that excessive nitrogen rendered plants more subject to attack by stem rust. After inoculation these plants were kept in a saturated atmosphere and consequently the leaves were covered with a film of moisture. Iljin (26) concludes from his experiments on the transpiration of wet leaves that immersion in water in the dark has the tendency to cause closure of the stomata. However, if potassium nitrate is present in the water, the stomata open.

Many investigators have shown that under certain conditions the amount of nitrate in the leaves of a plant supplied with an excess of this salt may be proportionately higher than in normal plants. Whether this condition can be associated with the findings of Iljin has not been determined.

Rust appeared only on plants in the nitrate series. It is possible that the environmental conditions during the incubation period of the series of January 29 were such that the stomata of all the plants except those growing in soil with high nitrogen content remained closed. This supposition is supported by the fact that, although the spores germinated profusely and abundant appressoria were formed on all plants, no flecks appeared on any of the plants which received no nitrogen, indicating that the lack of infection more likely was due to failure of germ tubes to enter than to any induced protoplasmic resistance. This explanation is partly confirmed also by the behavior of the plants in the second series. Here all plants became infected except those in series 125, which were grown in soil deficient in nitrogen. These plants were maturing early, the leaves were narrow, the stems upright, stiff, and provided with abundant bloom. The leaves, particularly, were rough and dry to the touch.

#### MORPHOLOGY OF HOST AS INFLUENCED BY NUTRIENT SALTS

In order to compare the morphology of plants of Haynes Bluestem wheat grown under different conditions of nutrition, sections of the leaves were cleared and stained with Pianezze stain. Four such sections are shown in Plate 2, K, L, M, and N. Here, L and N show typical sections of a leaf from a plant grown in abundant nitrogen, not balanced by other nutrients. The epidermis is composed of very large, thin-walled, noncutinized cells. There is very little sclerenchyma, and the fibers do not extend from one leaf surface to the other. The intercellular spaces are very large, the leaf being porous and succulent. Plate 2, K

and M, shows sections of a leaf from a plant grown in a culture deficient in nitrogen but containing an excess of phosphates. The contrast with Plate 2, L, is very marked. The epidermis, particularly on the lower surface, is composed of small, highly cutinized, thick-walled cells. Associated with the fibrovascular bundles is a relatively large amount of sclerenchyma, which extends from surface to surface. The chlorenchyma is compact and the intercellular spaces are small. The leaf is compact and firm. These sections were made from leaves of the same age, of the same wheat variety, and grown under the same conditions, the difference being only in the nutrient salts which the plants received.

Kraus and Kraybill (28) have shown that feebly vegetative tomato plants, grown in soil with a small amount of nitrogen, contain practically no nitrate nitrogen and but little total nitrogen. In these plants the dry matter and free reducing substance are comparatively high. The bast fibers and xylem tissues are greatly increased. On the other hand, plants growing under favorable vegetative conditions have higher total nitrogen and nitrate nitrogen and lower free reducing substance and dry matter. The bast and xylem tissues are comparatively greatly reduced.

Such studies suggest the possible function of potassium and sodium nitrate in the physiology of the plant. These salts appear to be concerned chiefly with carbohydrate utilization. If carbohydrates are not normally utilized there would tend to be an increase in the production of cell wall, crude fiber, and pentosans. The deficiency of nitrogen in the plants reported in these studies appears to result in thickened cell walls, as shown in Plate 1, D, and in an increase of fibers, as shown in Plate 2, L and M.

Plate 2, J, illustrates the typical restriction of rust development by the morphology of a wheat leaf. The sclerenchyma cells extend from the upper epidermis to the lower epidermis and permit the development of mycelium in only the longitudinal direction. Plowright (41) states that there is a tendency for the mycelium at the base of uredinia to spread in a centrifugal manner, but that many causes operate to prevent this, the chief being the lack of uniformity in the tissues of the host plant; and in a leaf with strongly marked venation this tends to exert a directive influence upon its extension. Eriksson and Henning (12, pl. 10, fig. 110) show a uredinium of *P. dispersa* on the seedling leaf of *Bromus* sp., and call attention to the concentric nature of the secondary uredinia, showing that the mycelium has spread in all directions from the original point of infection. The same condition occurs also in the wheat seedling infected with *P. graminis*. However, as figure 112 of the same plate shows, there is no such secondary infection on the older leaves. The sclerenchyma in the seedling leaf does not extend from epidermis to epidermis and, consequently, does not restrict the growth of the mycelium but permits it to grow in all directions. In the older leaves so many bundles of fibers have developed that the growth of the mycelium is restricted to the areas between the fibrovascular bundles.

Referring again to Plate 2, K, L, M, and N, it is possible to understand the influence of a nitrogenous fertilizer in increasing the size of the uredinia on the host plant. If the succulent leaf, Plate 2, L, becomes infected, the growth of the fungus would probably be rapid and extensive because there are no sclerenchyma fibers between the bundles and the epidermis to prevent its growth. The uredinia therefore would be large. But the growth of the distributive hyphae in such a leaf as that shown in Plate 2, K, would be restricted by the sclerenchyma fibers. Small

uredinia would be produced, because each one would be restricted to a single collenchyma bundle. There is likely to be relatively little sclerenchyma in leaves of plants which have been fertilized with an unbalanced excess of nitrogen; and the uredinia which develop on them therefore may be larger than those on plants which have been fertilized normally. While the actual protoplasmic resistance of the plants may not have been changed, there may be a greater total area of tissues in which the rust mycelium can grow.

Furthermore, heavy nitrogenous fertilization promotes rank growth and dense stands of wheat. Moisture, therefore, is retained and the opportunities for spore germination are increased. An excess of nitrogen also seems to increase the transpiration of the plants, causing the stomata to open and thus facilitate the entrance of the germ tubes.

The balance of nutrients seems to be much more important than the total amount. The results of field experiments on the effect of fertilizers on rust development are therefore significant only if the soil type is definitely known.

#### THE RELATION OF PHYSICOCHEMICAL PROPERTIES OF THE PLANT SAP TO RUST RESISTANCE

As some varieties of wheat are completely susceptible to certain biologic forms of *P. graminis tritici* and are immune from others, it is obvious that the sole basis of resistance can not be morphological characters. It is a well established fact, for instance, that Kanred is completely susceptible to some biologic forms and it is so highly resistant to others that not even flecks are developed on plants inoculated artificially. It is known, also, that the mycelium of the biologic forms to which Kanred is resistant can not develop extensively within the tissues. Therefore, there is a real protoplasmic or physiologic resistance. The exact nature of this resistance has never been determined. Hurd (24) studied the possible relation of hydrogen-ion concentration of wheat varieties to their resistance to rust and other pathogenic fungi, and concluded that hydrogen-ion concentration probably had little effect on resistance. In fact, the concentration varied more in the same variety grown under different conditions than it did in different varieties.

The osmotic concentration of the sap of a host plant probably exerts considerable influence on the ability of the rust fungus to absorb nutriment from it. The total solids indicate the amount of moisture in the plant tissues and therefore to a certain extent are correlated with the succulence of the plant. It is possible, therefore, that differences in total solids might affect the development of rust. The importance of available carbohydrates in the nutrition of rust fungi has been shown clearly by Mains (33). Henning (22) and Kirchner (27) were of the opinion that the content of reducing sugars was lower in rust-susceptible than in resistant varieties. Eckerson (11) has shown that the carbon substances vary in relative amount in the plant sap at different stages of development of the wheat plant.

The writer made attempts to ascertain whether there were differences in the physicochemical properties of different varieties great enough and sufficiently consistent to account for differences in their resistance to rust. Particular attention was paid to the determination of the depression of the freezing point, total solids, average molecular weight, hydrogen-ion concentration, and sugar content. The following methods were

used: Seedlings of the different varieties were frozen in carbon dioxide and pulverized while still frozen. The powdered plant tissue was thawed at room temperature and the sap then extracted by pressure. Freezing-point depression was determined by means of a Beckmann thermometer. Total solids and average molecular weight of solutes were determined by the refractometric method described by Gortner and Hoffman (18). A Leeds and Northrup type (K) potentiometer was used in securing the hydrogen-ion values. The results of these determinations are given in Table X. For analysis of sugars present in the sap, seedlings were grown under continuous light.<sup>4</sup> Samples of 25 gm. each, green weight, were extracted in methyl alcohol, and total and reducing sugars determined by the picric acid method described by Rose (45). Dry weight was determined from separate samples dried at 65° in vacuum. The results of sugar determinations are given in Table XI.

TABLE X.—*Physicochemical properties of the sap of six wheat varieties*

Wheat variety.	Freezing-point depression.	Refractometric solids.	Average molecular weight of solutes.	PH.	Relative susceptibility. <sup>a</sup>
	° C.				
Khapli, C. I. 4013.....	0.95	8.50	173	5.984	2.2
Mindum, C. I. 5296.....	0.98	.....	.....	6.194	41.2
Kota, C. I. 5878.....	0.91	7.55	177	6.062	40.1
Kanred, C. I. 5146.....	1.08	8.40	159	5.890	32.8
Marquis, C. I. 3641.....	1.02	7.00	138	5.973	60.8
Little Club, C. I. 4066.....	0.82	6.46	155	5.899	88.4

<sup>a</sup> 100=completely susceptible. Unpublished computations of E. C. Stakman and M. N. Levine on the basis of reaction of wheat varieties to all known biologic forms of *P. graminis tritici*. Little Club, while susceptible to all biologic forms, does not always produce the most virulent type of infection.

TABLE XI.—*Determination of sugars in sap of six wheat varieties*

Wheat variety.	Reducing.		As sucrose.		Total.	
	Percentage of green weight.	Percentage of dry weight.	Percentage of green weight.	Percentage of dry weight.	Percentage of green weight.	Percentage of dry weight.
Khapli, C. I. 4013.....	0.6856	7.4072	0.1420	1.5176	0.8276	8.9248
Mindum, C. I. 5296.....	.6857	7.3896	.2376	2.5704	.9242	9.9600
Kota, C. I. 5878.....	.7680	8.3296	.0809	0.5312	.8489	8.8602
Kanred, C. I. 5146.....	.8531	8.9392	.0000	0.0000	.8531	8.9392
Marquis, C. I. 3641.....	.7515	8.0992	.0000	0.0000	.7515	8.099
Little Club, C. I. 4066.....	.7315	8.6544	.0000	0.0000	.7315	8.654

Table X shows that there are some differences in the physicochemical properties of different wheat varieties. There is a smaller percentage of solid matter in the sap of Little Club than in that of the other varieties. This is indicated both by the freezing-point determination and refractometric methods. There appears to be no correlation, however, between

<sup>4</sup> The plants were grown in one of the constant-light rooms described by Harvey (21).

the amount of solids in the sap and rust resistance. The hydrogen-ion concentrations of the different varieties differed but little, an observation agreeing with that of Hurd (24). The data presented in Table X show that there is no consistent correlation of any of the sap properties of different varieties and their relative degree of resistance to biologic forms of *Puccinia graminis tritici*.

The data given in Table XI indicate that, under the conditions of the experiment, varieties show a difference in the amount of both reducing sugar and total sugars. No sucrose was present in Little Club, Marquis, and Kanred, all of their sugars analyzing as reducing sugar. These varieties also contained higher concentrations of reducing sugars than did Khapli and Mindum. In Khapli and Mindum 17 and 25.8 per cent, respectively, of the total sugar was sucrose. Khapli is resistant to all of the biologic forms described by Stakman and Levine; Mindum is resistant to some and susceptible to others. From the data presented in Table XI, we are not justified in drawing any definite conclusions as to the relation of sugar content to rust development. It can be pointed out that wheat varieties do differ considerably in their sugar content and that detailed investigations may give significant information both on the general nature of the relation of rust development to sugar content of the host sap and to the difference in behavior of biologic forms. Such an analysis is of little value, however, without a complete analysis of the carbon metabolism, changes in plant acids, etc.

The difficulty in attempting to explain the nature of resistance to stem rust on the basis of specific physicochemical properties of the sap of a variety is because of the difference in behavior of a particular variety to a number of biologic forms of *Puccinia graminis tritici*. Khapli is quite resistant to all of the biologic forms studied by Stakman and Levine and it is conceivable, though not proved, that this resistance may be due to certain physicochemical properties.

Little investigation has been made to determine physiological differences between biologic forms of *P. graminis tritici*. The writer previously has reported that a distinct physiological difference can be demonstrated in biologic forms apart from the differential hosts used in identifying them. It was shown (25) that urediniospores of two biologic forms germinate differently under different conditions of temperature and hydrogen-ion concentration. Furthermore, the urediniospores of a biologic form which attacks only a few differential hosts could not germinate well under such a wide range of environmental conditions studied as could those of a biologic form which can attack many hosts. That is to say, the potentiality or possibility for germ-tube development is greater in the biologic form with a wide host range. An organism restricted in its activity to a narrow temperature range might possibly also have a narrow range of adjustment to other environmental variables. In seeking for the significance of this fact in relation to rust resistance, however, we must turn to the physiology of the host.

We know that there are variations in the morphologic and physiologic characters of varieties within a species. Vavilov (56) has concluded that these variations may occur as homologous series within related taxonomic groups. It is probable, therefore, that there also are physiologic variations. Since resistance is in part physiological in nature, or expresses itself as such, we see that different wheat varieties present to a rust organism a range of physiologic reaction with which it must compete successfully if it is able to infect. It would appear, then, that a

biologic form which has the potential ability to adjust itself to a wide range of conditions or reactions might be able to maintain itself in a greater number of host varieties than would a biologic form with limited range of tolerance to certain physiologic reactions.

In the same way there may be corresponding differences in the physiology of biologic forms of *P. graminis*, and this may account even for the existence of biologic forms. Then it would naturally follow that forms differ in ability to adjust themselves to physiologic environment.

It is reasonable to expect that all biologic phenomena are ultimately to be explained on a physicochemical basis. The action of poisons and toxins are not exceptions. A conception of biologic forms based on physiological studies, as has just been indicated, would be quite in keeping, therefore, with the accepted ideas of fundamental resistance.

### GENERAL CONCLUSIONS

It is obvious that the basis for resistance of wheat varieties to biologic forms of *P. graminis tritici* must be either morphological or physiological. For a long time it was supposed that the basis of this resistance might be morphological. The work of Ward, however, indicated that external morphological characters probably were of minor importance in determining resistance or susceptibility of plants to the attack of rust fungi. More recently still, however, the work of Allen and others, together with extensive field observations, has called attention to the fact that morphological characters might play some part in determining resistance. It has been suggested that the morphology of the plant might affect resistance by preventing the entrance of germ tubes.

It seems reasonable to suppose that certain morphologic characters might possibly interfere with the entrance of germ tubes. As the germ tubes of the urediniospores always enter the wheat plant through stomata, it would seem especially reasonable to suppose that the number, distribution, size, and location of the stomata might be quite important in determining how many germ tubes could enter the plant. Furthermore, we may assume that the number of hairs on the plant surface may have some effect on the growth of the germ tubes, as an abundance of hairs easily may block the growth of the tubes. Extensive observations were made on the number of hairs and the number and size of the stomata on susceptible and resistant varieties of wheat. While the number of hairs in general was greater on the more resistant varieties than on the more susceptible ones, it seemed evident that the number of hairs scarcely could be a determining factor in the entrance of the germ tubes. The number of stomata, as well as the size of the stomatal aperture, differs for different varieties. However, some of the most resistant hosts have stomata with large apertures, while some very susceptible ones have stomata with small apertures. It seems probable that the stomatal aperture of all varieties is sufficiently large to permit the entrance of urediniospore germ tubes. It seems much more likely that the character of the stomatal movements, which may differ in different varieties, would have more effect upon entrance. The size of the stomatal opening scarcely could account for the resistance of certain varieties to certain biologic forms, because some varieties are immune from certain biologic forms and completely susceptible to others. It never has been demonstrated that the size of germ tubes of the different biologic forms differs sufficiently to make an explanation of resistance on this basis of entrance

seem reasonable. It is quite true that appressoria may be produced fairly abundantly on the surface of certain plants, without any subsequent development of the germ tubes. The failure of the fungus to enter, even after appressoria have been formed, probably must be explained on the basis of the absence of the necessary stimulus or possibly by the failure of stomata to remain open sufficiently long to enable the rust fungus to enter.

There can be no question whatever but that there is a real physiologic or protoplasmic resistance. For instance, Khapli is resistant to all biologic forms of *P. graminis tritici*, though flecks practically always develop after inoculation. Naturally, therefore, the fungus must have entered the host tissue. It has been demonstrated beyond doubt that the cells of many resistant hosts are hypersensitive to the attacks of the fungus (2), (34), (51). They are killed very quickly after the fungus comes in contact with them, and the hyphae of the invading fungus then die.

It has been claimed that the physiology of the host plant can be changed rapidly and profoundly enough by altering environmental conditions to cause great variations in the protoplasmic resistance of the host to *P. graminis*. While this never has been demonstrated, certain observed facts seem to substantiate the claim. It frequently has been observed that the resistance of a variety apparently may change when conditions are altered. It also has been shown that young plants sometimes are more susceptible than older ones. For instance, Stakman and Piemeisel (55) have observed that young plants of *Agropyron smithii* can be infected easily as a result of artificial inoculations. It is much more difficult, however, to infect older plants. It also has been observed that certain varieties of wheat, such as Kota and Acme, are susceptible to a considerable number of biologic forms in the greenhouse but that they seem to be fairly resistant in the field. This fact must be due either to physiologic or morphologic changes in the host.

Extensive studies of the morphology of different varieties of wheat were made in order to ascertain whether there were any differences sufficiently great to account for the differences in rust resistance. It was found that the amount of sclerenchymatous tissue in the stem of Kota, Acme, and certain other varieties is very large in proportion to the amount of the chlorenchymatous collenchyma. As the rust can live practically only in the chlorenchyma, and as the only tissue in the stem which contains chlorophyll is the collenchyma, it is clear that the amount of collenchyma would determine to a very considerable extent the amount of development which is possible for the rust fungus. In certain resistant varieties the sclerenchymatous tissues have developed to such an extent as to decrease considerably the amount of space in which the rust can grow. Sclerenchymatous strands extend from the vascular bundles to the epidermis and a complete sclerenchyma sheath extends around the stem just outside the vascular bundles. The collenchyma bundles, therefore, are small and completely separated from each other. They are surrounded on three sides by thick-walled, lignified, sclerenchymatous fibers, through which the rust hyphae can not grow and from which they can not obtain nourishment. The size of the uredinia, therefore, is limited by the size of the collenchyma bundles. It is impossible for the rust to spread for any distance tangentially or radially. It can spread only longitudinally and this probably accounts for the fact that long,



narrow uredinia often are formed on varieties which appear to be generally resistant in the field.

The morphological structure of a stem can be changed by environmental conditions. It has been shown that the ratio of sclerenchymatous tissue to collenchymatous tissue can be reduced by unbalanced nitrogen nutrition of the plant. The effect of large amounts of nitrogenous fertilizers, therefore, especially when their effect is not counterbalanced by the addition of other fertilizers, probably is to increase the area in which the rust fungus can grow. In this way, fertilizers appear to sensitize plants to infection. We should not overlook the fact, however, that the fundamental protoplasmic resistance of the plant probably has not been changed. The individual cells probably are just as susceptible in plants fertilized with nitrogen as they are in those which have not been so fertilized, but the number of cells in which the rust can develop is greater. Conversely, phosphates and potassium fertilizers may cause an increase in the amount of sclerenchymatous tissues and thus limit the area in which the rust mycelium can grow.

It is quite likely also that the reason why seedlings sometimes seem to be more susceptible than older plants is that the morphology of the seedlings is somewhat different from that of the older plants. It has been observed frequently that varieties which are susceptible in the seedling stage behave in a peculiar manner in the field. Either very few uredinia are produced on the plants in the field or they remain small. The explanation for this fact probably is that the amount of sclerenchymatous tissue in proportion to the other tissues is greater in the stems of older plants than in the leaves of seedlings. While the basic resistance, therefore, of the plant is not different from what it was when the plant was young, still the area in which the rust can develop is limited mechanically.

No satisfactory explanation ever has been given for the basic or protoplasmic resistance of wheat varieties. It seems quite obvious that this must be due to physicochemical relations between the host and the pathogene. As the pathogene can not be grown in artificial culture media, a study of the problem is somewhat difficult. The most promising method of attack would seem to be to ascertain whether there are consistent differences in the physicochemical properties of different varieties. Determinations therefore were made of hydrogen-ion concentration, sugar content, etc. None of the observed differences seemed to be consistent or great enough to account for the differences in resistance. While it may be significant that the sugar concentrations in different varieties varied somewhat, too much importance should not be attached to these results. A study also was made of the reaction of spores of different biologic forms to certain physicochemical factors. It was found that the spore germination of two biologic forms was affected differently by hydrogen-ion concentration and by temperature. The form with the widest host range was able to withstand a wider range of variation than was the form with the narrower host range. This fact probably is of some significance, but just how much it is impossible to say.

While the question of the fundamental nature of resistance of wheat varieties to *P. graminis* was not completely solved, it at least has been shown that many of the rather puzzling and conflicting observations on apparent change of resistance can be explained easily on the basis of morphologic changes which occur within the host. It should be kept clearly in mind, however, that this is not a change in the fundamental



resistance but only a change in the opportunities of the rust to develop, due to a spatial limitation of the growth of the organism.

#### SUMMARY

1. In addition to fundamental protoplasmic resistance, wheat varieties may possess other means of defense against *Puccinia graminis*.
2. The number of leaf hairs and the size and number of the stomata can not be considered important in influencing the entrance of the germ tubes. When there is only a small amount of inoculum, the large number of hairs on some varieties may prevent some of the germ tubes from reaching the stomata and growing through the stomatal slits.
3. Stomatal movements may have some influence on the entrance of germ tubes. The stomatal movements of different varieties of wheat apparently are affected differently by environmental conditions.
4. The mycelium of *P. graminis* within the host is limited almost entirely to chlorenchymatous tissue. As the only important chlorenchymatous tissue of the stem is the collenchyma, the rust mycelium can grow only in this tissue. In some varieties of wheat there is such a large amount of sclerenchyma that the band of collenchymatous tissue is broken up into small bundles. The extent of mycelial development, therefore, is limited to these relatively small areas.
5. The amount of sclerenchyma is not the same in the stems of different varieties. In some there is a very large amount and in others relatively little. The amount of collenchyma is approximately inversely proportional to the amount of sclerenchyma. In those varieties, therefore, in which there is a large amount of collenchyma, large uredinia are likely to be produced, while in those varieties in which the collenchyma bundles are small, the uredinia are likely to be narrowly linear. Varieties in which there is a great deal of sclerenchyma are likely to be injured less by rust, as there is a mechanical limitation to the spread of the mycelium.
6. The relative proportion of sclerenchyma to collenchyma in a given variety may be altered by the use of fertilizers.
7. Excessive fertilization with nitrogen has a tendency to decrease the amount of sclerenchyma in proportion to the amount of collenchyma. For this reason, plants heavily fertilized with nitrogen may be more severely injured by rust than those which have not been so fertilized.
8. The fact that the seedlings of some varieties appear to be more susceptible to certain biologic forms of *P. graminis* than are the older plants can be explained by differences in morphology between the seedlings and the mature plants. There is a greater amount of sclerenchyma in the mature plants than in the seedlings and this constitutes a mechanical restriction on the growth of the mycelium.
9. There are differences in the physicochemical properties of the sap of different wheat varieties. It has been impossible, however, to make a definite correlation between these properties and rust resistance.
10. The differences in the reaction of wheat varieties to different biologic forms of *P. graminis tritici* appear to be due entirely to physiologic causes.

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# PLATE 1

A.—Strip from epidermis of stem of Little Club wheat, C. I. 4066, showing relative number of stomata per unit area.

B.—Strip from epidermis of stem of Marquis wheat, C. I. 3641, showing relative number of stomata per unit area.

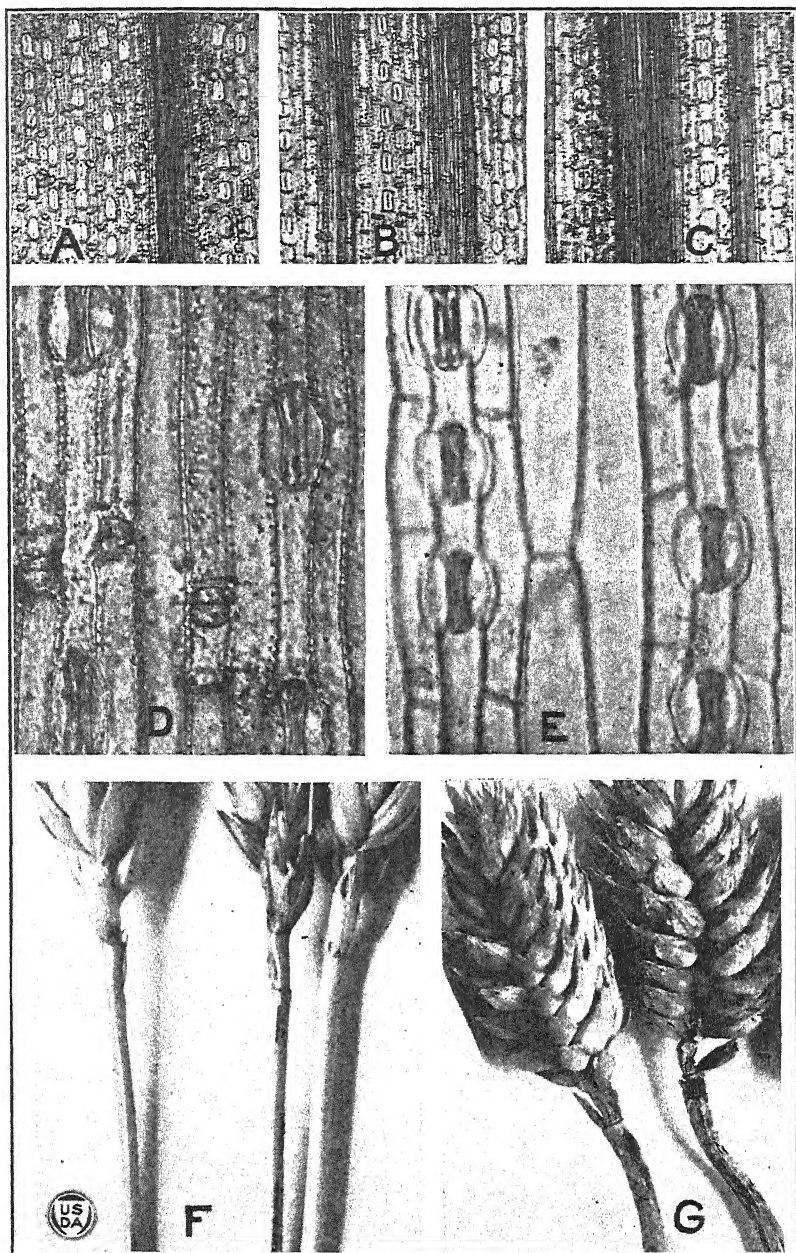
C.—Strip from epidermis of stem of Kota wheat, C. I. 5878, showing relative number of stomata per unit area. All  $\times 75$ .

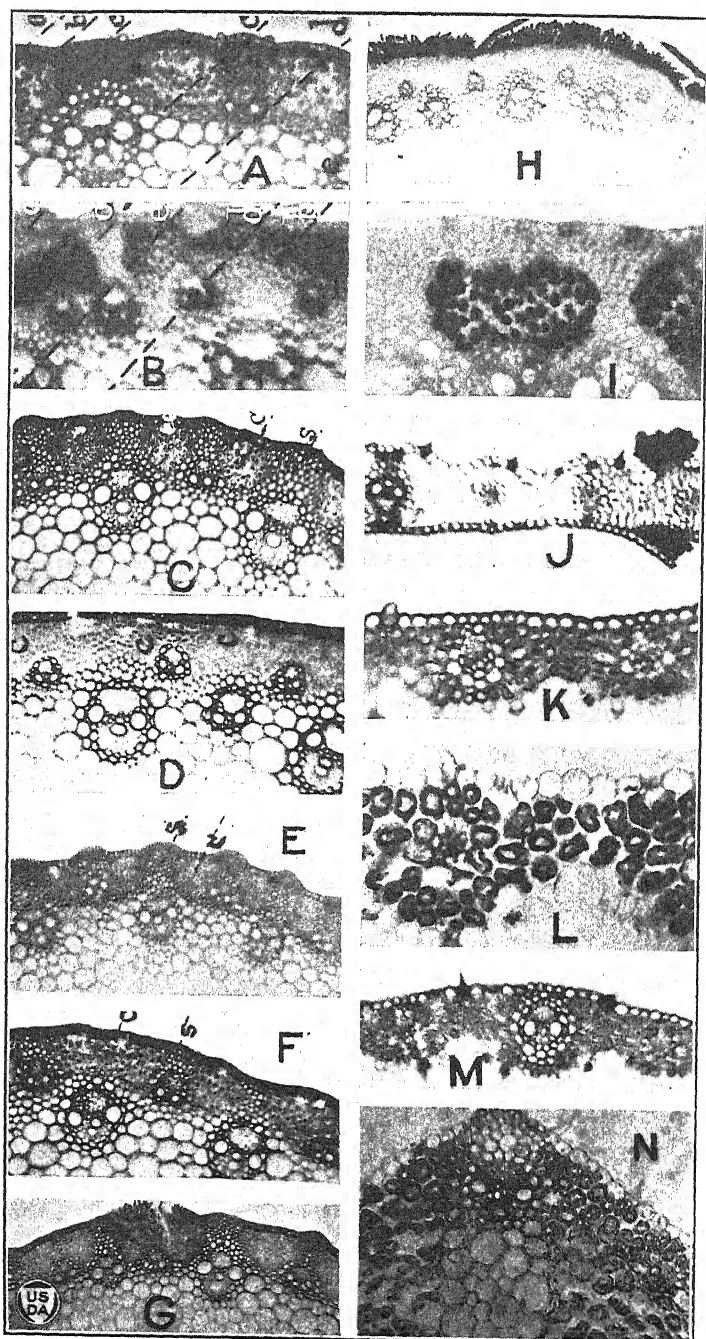
D.—Typical epidermis from plant of Haynes Bluestem wheat grown in nitrogen-deficient culture.

E.—Typical epidermis from plant of the same variety grown in culture supplied with excess nitrogen.

F.—Portions of heads and peduncles of Kota wheat, C. I. 5878, showing solitary linear uredinia on peduncle.

G.—Heads and peduncles of Little Club wheat, C. I. 4066, showing confluent, oblong uredinia.







## PLATE 2

A.—Section of wheat stem showing a moderate amount of sclerenchyma fiber development. The tissues are as follows: (a) Epidermis, (b) collenchyma, (c) sclerenchyma fibers ( $\times 120$ ), (d) thin-walled parenchyma, (e) fibrovascular bundles.

B.—Section of wheat stem showing a relatively small amount of sclerenchyma development in proportion to collenchyma. The tissues are lettered as in A.

C.—Cross section of upper part of peduncle of Kota wheat, C. I. 5878. The collenchyma (c) is restricted to small unit areas separated by the sclerenchymatous fibers (s).  $\times 60$ .

D.—Cross section of upper part of peduncle of Little Club wheat, C. I. 4066. The collenchyma (c) is almost continuous.  $\times 60$ .

E.—A similar section from the peduncle of Marquis wheat, C. I. 3641, showing a moderate amount of sclerenchymatous tissue (s) in proportion to the collenchyma (c).  $\times 60$ .

F.—Cross section of peduncle of Sonem emmer, C. I. 4402. There is a large amount of sclerenchymatous tissue (s) in proportion to the collenchyma (c).  $\times 60$ .

G.—Transverse section of peduncle of Kota wheat, C. I. 5878, infected with *Puccinia graminis*. Note the small uredinium confined to a single collenchyma bundle. Lateral spread of rust is impossible because of the strands of sclerenchymatous tissue.  $\times 45$ .

H.—Similar section of peduncle of Little Club wheat, C. I. 4066. Note the very large uredinium which probably has resulted from the confluence of several smaller ones. Lateral spread of infection is not restricted by sclerenchyma.  $\times 45$ .

I.—Transverse section of the upper peduncle of Acme wheat, C. I. 5284, showing an almost continuous band of sclerenchyma just under the epidermis.  $\times 200$ .

J.—Transverse section of a portion of a typical mature leaf of Haynes Bluestem wheat showing restriction of rust mycelium to the chlorenchyma between two fibrovascular bundles.  $\times 75$ .

K.—A similar leaf section from a plant of Haynes Bluestem grown in nitrogen-deficient soil.  $\times 60$ .

L.—Transverse section of a leaf of Haynes Bluestem plant grown in soil containing excess nitrogen.  $\times 60$ .

M.—Transverse section through the midrib of a leaf of a Haynes Bluestem plant grown in nitrogen-deficient soil.  $\times 45$ .

N.—A similar section through the midrib of a leaf of a Haynes Bluestem plant grown in soil containing excess nitrogen.  $\times 45$ .



# THE FUNCTION OF GRIT IN THE GIZZARD OF THE FOWL<sup>1</sup>

By B. F. KAUFF

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## INTRODUCTION

As a preliminary step in the study of the nutrition of fowls, it is necessary to determine the function of grit in the gizzard and the length of time that it will remain there and serve a useful purpose. A review of the literature of the subject discloses no record of experiments made to discover these facts.

## THE PROBLEM

It is a matter of common knowledge that, since the fowl has no teeth with which to grind its food, the muscular walls of the gizzard contract upon its contents and reduce the food to fineness. The object of this investigation was to discover how long such grit is useful in the gizzard, how often it must be replenished, whether a hen constantly consumes more grit than she requires, and if so, whether the surplus is kept in the gizzard.

## EXPERIMENTAL METHODS

Barred Plymouth Rock hens 2 or 3 years old were used in the experiment. They were kept in coops 18 inches square. The coops were provided with 1-inch mesh wire bottoms so that the excreta would pass through to a second floor as soon as voided. A possible reconsumption of any grit passed in the excreta was thus prevented. Hens were killed at different periods and the gizzard content examined for the grit which still remained.

Analyses of the intake and outgo of the feed and the weight of the birds were made to determine whether or not the grit content of the gizzard was sufficient for the normal physiological processes of that organ.

The feed for 365 days, the duration of the test, consisted of the regular scratch feed and dry mash used at the Station plant. The following tabulation offers a comparative study of the amount of grit contained in the gizzards of hens killed at different intervals of time. This information is differently presented in Plate 1.

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TABLE I.—*Grit remaining in the gizzards of fowls at the end of different periods*

Hen No.	Number of days without grit.	Weight of hen at beginning of experiment.	Weight of hen when killed.	Weight of grit in gizzard.	Remarks.
		<i>Pounds.</i>	<i>Pounds.</i>	<i>Gm.</i>	
1 .....	14	5.2	5.4	9.5012	Killed.
2 .....	14	6.4	6.3	13.1136	Do.
3 .....	21	4.5	4.8	8.3126	Do.
4 .....	21	5.3	5.8	16.9326	Do.
5 .....	28	4.6	4.9	11.8763	Do.
6 .....	28	7.0	7.3	22.6531	Do.
7 .....	36	4.8	5.7	16.4389	Do.
8 .....	36	2.7	4.0	5.0378	Do.
9 .....	42	7.0	4.2	8.4531	Died of sarcoma.
10 .....	79	4.6	4.7	6.3700	Killed.
11 .....	93	3.6	3.2	11.6341	Do.
12 .....	120	3.8	6.2	4.9643	Do.
13 .....	124	5.8	6.1	5.6321	Do.
14 .....	133	5.6	4.9	4.5032	Killed by mites.
15 .....	134	7.0	5.8	4.7532	Killed.
16 .....	144	5.7	5.7	9.5920	Killed by mites.
17 .....	153	7.1	5.6	6.5120	Do.
18 .....	154	6.5	5.5	5.9633	Killed.
19 .....	156	6.8	5.8	14.0326	Killed by mites.
20 .....	170	(?)	(?)	9.8070	Died. <sup>a</sup>
21 .....	248	6.2	6.1	2.5200	Killed.
22 .....	270	6.3	6.6	5.0000	Do.
23 .....	300	5.7	5.9	3.9525	Do.
24 .....	330	5.1	6.1	1.9530	Do.
25 .....	365	6.2	5.2	2.5610	Do.
26 .....	395	6.7	7.1	5.8915	Do.

<sup>a</sup> This was a cockerel affected with partial paralysis from which it never entirely recovered. It was sent to the laboratory when it weighed about 2 pounds, and remained in the coop until it died, 170 days later.

## DISCUSSION

The feed records show that the appetite of the birds having no exercise kept up fairly well.

From the experiments herein recorded it is apparent that a bird may go 365 days without grit being fed to it and still have enough remaining in its gizzard to grind its food. The grit that remained in the gizzard for 365 days appeared just as sharp as that found at the beginning of the experiment. In fact, the writer does not believe that the grinding in the gizzard of the fowl is a sharp-cutting process. Rather it appears that the food soaks more or less in the crop, depending on the length of time it remains there. It then passes from the crop through the second portion of the esophagus to the proventriculus, where it lies in an acid secretion. From the proventriculus it passes into the gizzard and there the muscles of the walls contract, forcing the soaked grain among the particles of grit and by a squeezing rotary motion reduces it to fineness. The action is like that of a ball mill. Birds hold their weight and remain perfectly healthy on either sharp or dull grit.

There is a tendency on the part of fowls to eat more grit than is essential for grinding their food. In another series of experiments the writer has found that the amount of mineral given off for the first twelve days was much greater than that taken in. Further experiments showed that this was due to the grit passed off from the gizzard. While

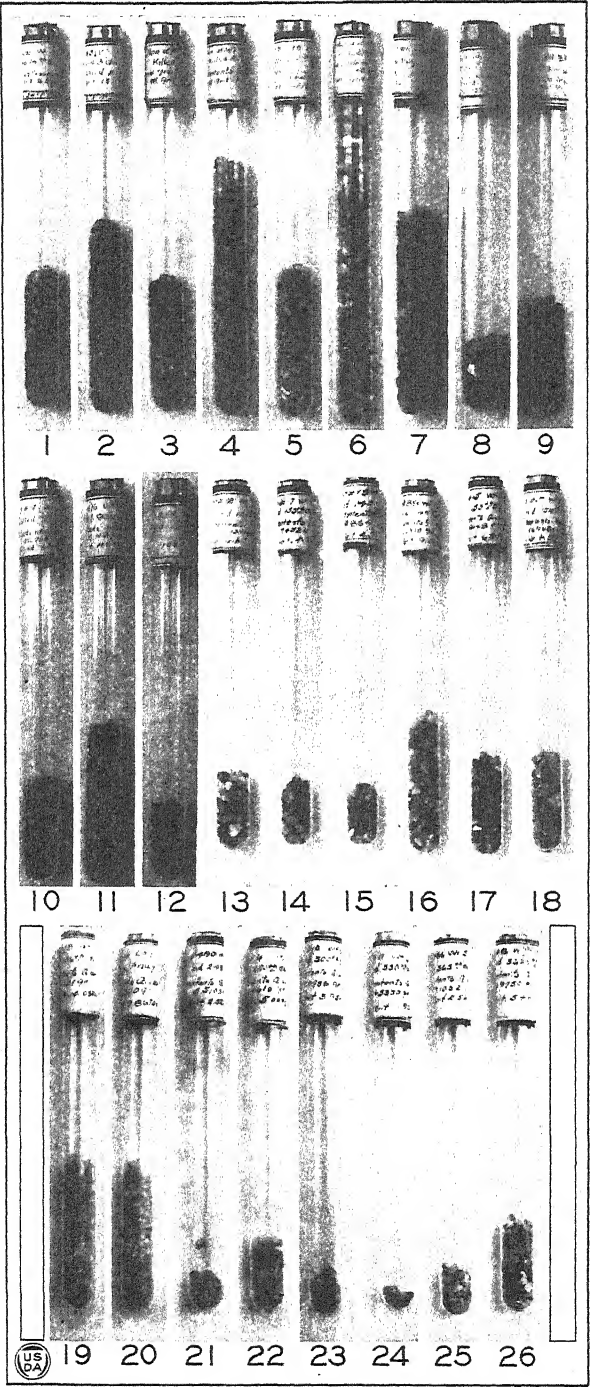
there is a tendency to pass off the excess grit and to keep a residual amount, the amount retained varies greatly in different individuals.

At the end of 365 days the gizzard of one hen contained 5.89 gm. of grit—as much as was found in a hen killed for examination on the thirty-sixth day of the experiment. A hen that died on the one hundred and fifty-sixth day had 14.03 gm. of grit, or more than that contained in three of the four gizzards of those killed on the fourteenth and twenty-first days, respectively. The healthy condition of the birds which were kept until the end of the experiment indicates that a fowl may go longer than a year without replenishing its grit.

# PLATE I

The amount of grit recovered from the gizzards of the fowls at the end of the experimental periods:

- 1.—Hen killed 14th day; weight of gizzard, 13 gm.; weight of grit, 9.50 gm.
- 2.—Hen killed 14th day; weight of gizzard, 21 gm.; weight of grit, 13.11 gm.
- 3.—Hen killed 21st day; weight of gizzard, 12.01 gm.; weight of grit, 8.31 gm.
- 4.—Hen killed 21st day; weight of gizzard, 19.03 gm.; weight of grit, 16.93 gm.
- 5.—Hen killed 28th day; weight of gizzard, 13.46 gm.; weight of grit, 11.87 gm.
- 6.—Hen killed 28th day; weight of gizzard, 28.19 gm.; weight of grit, 22.65 gm.
- 7.—Hen killed 36th day; weight of gizzard, 19 gm.; weight of grit, 16.43 gm.
- 8.—Hen killed 36th day; weight of gizzard, 7.10 gm.; weight of grit, 5.03 gm.
- 9.—Hen killed 42d day; weight of gizzard, 14.13 gm.; weight of grit, 8.46 gm.
- 10.—Hen killed 79th day; weight of gizzard 20.76 gm.; weight of grit, 6.37 gm.
- 11.—Hen killed 93d day; weight of gizzard, 15.43 gm.; weight of grit, 11.63 gm.
- 12.—Hen killed 120th day; weight of gizzard, 9.63 gm.; weight of grit, 4.96 gm.
- 13.—Hen killed 124th day; weight of gizzard, 14.13 gm.; weight of grit, 5.63 gm.
- 14.—Hen killed 133d day; weight of gizzard, 7.45 gm.; weight of grit, 4.56 gm.
- 15.—Hen killed 143d day; weight of gizzard, 9.86 gm.; weight of grit, 4.75 gm.
- 16.—Hen killed 144th day; weight of gizzard, 13.23 gm.; weight of grit, 9.59 gm.
- 17.—Hen killed 153d day; weight of gizzard, 11.96 gm.; weight of grit, 6.51 gm.
- 18.—Hen killed 154th day; weight of gizzard, 11.76 gm.; weight of grit, 5.96 gm.
- 19.—Hen killed 156th day; weight of gizzard 17.36 gm.; weight of grit, 14.03 gm.
- 20.—Hen killed 170th day; weight of gizzard, 14.93 gm.; weight of grit, 9.86 gm.
- 21.—Hen killed 248th day; weight of gizzard, 4.51 gm.; weight of grit, 2.52 gm.
- 22.—Hen killed 270th day; weight of gizzard, 10.46 gm.; weight of grit, 5 gm.
- 23.—Hen killed 300th day; weight of gizzard, 10.09 gm.; weight of grit, 3.95 gm.
- 24.—Hen killed 330th day; weight of gizzard, 6.53 gm.; weight of grit, 1.93 gm.
- 25.—Hen killed 365th day; weight of gizzard, 7.10 gm.; weight of grit, 2.56 gm.
- 26.—Hen killed 365th day; weight of gizzard, 9.97 gm.; weight of grit, 5.89 gm.







# THE ASSOCIATION OF MANGANESE WITH VITAMINS <sup>1</sup>

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In a former paper (7)<sup>2</sup> data were presented to show that manganese is an essential element in the growth and normal development of autotrophic plants and functions in the synthesis of chlorophyll. The purpose of this paper is to present data which further confirm previous conclusions as to the essential character of manganese as a vital factor in life processes, to suggest a new theory concerning the nature of the vitamin factors, and to present data in support of that theory.

The observations of Takaki and the experiments of Eijkman, as quoted by Sherman and Smith (11, p. 8-9), show conclusively that the pericarp and germ of rice and barley grains contain a vital factor which prevents the development of beriberi in animals when the brown unpolished grains are consumed in the diet.

As early as 1854 Liebig (4) called attention to the fact that in the modern process of milling wheat the resulting flour contains less nutritive value than flour made from the whole grain. He states that "No single foodstuff loses its value so readily as whole grain through the modern process of milling. The whiter the flour the less nutritive value it possesses." These statements have been confirmed in many ways since the time of Liebig, and it is apparent that the pericarp and germ of wheat, barley, and rice each contains some unidentified vital factor which is necessary for the normal metabolic processes and physiological well-being in animal life.

Physiologists and biochemists everywhere are strenuously endeavoring to ascertain the function of each of the known chemical constituents of food. Since manganese is a known constituent of foods, the question arises as to whether or not this element has important functions in the metabolic processes of animal life which have been overlooked in previous investigations.

In 1914 the writer (5) published a paper in the *Journal of the American Chemical Society*, entitled "The Occurrence and Significance of Manganese in the Seed Coat of Various Seeds." In this paper it was shown that the pericarp of wheat and of a considerable number of other seeds contains much more manganese than the starchy and glutinous portions of the endosperm. It was suggested at the conclusion of the article that probably the manganese contained in the pericarp of different seeds performs an important function in the growth of plants. While this suggestion was somewhat prophetic at the time, the author has since obtained conclusive evidence that manganese does play the part of an essential element in plant growth. This fact is evidenced in data previously published (6, 7) and is further confirmed by the accompanying photograph of tomato plants (Pl. 1), the fruit of which is assumed to contain an important vitamin factor.

<sup>1</sup> Accepted for publication Nov. 19, 1923. Contribution from the Laboratory of Chemical Research of the Kentucky Agricultural Experiment Station. Published with permission of the Director.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 424.

Experiments conducted with many additional species of plants establish the fact that manganese is an essential element for the metabolic processes and the normal growth of autotrophic plants. When manganese becomes a limiting factor in the growth of plants, one of the first effects to be noted is a chlorotic condition of the young leaves and buds as they unfold—a fact which shows that this element has a function in the vital processes and is necessary for the synthesis of chlorophyll. The green leaves of plants are known to be an important source of vitamins, whereas blanched or chlorotic leaves are poor in vital factors; it has been shown also that green leaves contain more manganese than any other part of the plant, thus indicating that an interrelationship exists between chlorophyll, vitamins, and manganese.

#### MANGANESE IN THE PERICARP OF SEEDS

Since it has been demonstrated that the vitamin in whole grain of wheat, barley, and rice is contained in the pericarp and the germ, an examination of these cereals was made to determine the amount of manganese in the different parts of each and to show what proportion is removed in the modern processes of milling. Accordingly, samples of rice bran, rice polishings, unpolished rice grains, and polished white rice grains were analyzed for manganese. The results obtained, in parts per million of the air-dry material, are as follows:

Material:	Manganese
Rice bran.....	350
Rice polishings.....	100
Unpolished rice grains.....	25
Polished rice grains.....	10

From these results it is apparent that rice bran contains the largest quantity of manganese and the polishings contain the next largest, which is less than one-third of the amount found in the bran. The unpolished rice grains contain only one-fourteenth as much manganese as the bran and one-fourth as much as the polishings but two and one-half times as much as that found in the polished white rice. It is therefore apparent that in the process of polishing rice the greater part of the manganese is removed from the grain. It is logical to assume that the manganese contained in the pericarp and germ, and removed in the polishing, is in a different and perhaps more active combination than that contained in the more starchy and glutinous part of the endosperm. This assumption finds support in the results obtained by Eijkman (*11*, p. 8-9) and others who have found that the addition of the pericarp of the rice grain or even the bran to polished white rice prevents the development of beriberi in experiments with animals.

It has been shown by many investigators that the vitamin contained in rice bran and polishings is soluble in water, but less so in 95 per cent alcohol. The writer has examined the aqueous and alcoholic extracts from rice bran and polishings and has found that a considerable proportion (80 parts per million) of the manganese in each of these materials is soluble. The amount removed by alcohol depends upon the percentage of water contained in the alcohol. Very small amounts of manganese are soluble in 95 per cent alcohol, and the amount dissolved increases as the percentage of water in the alcohol increases, a fact in harmony with the findings that the aqueous and alcoholic extracts of rice bran and rice polishings contain the so-called vitamins.

## BARLEY

The pericarp of the barley grain is slightly less pronounced than is that of unpolished rice; however, upon close observation, its presence can be detected. Five gm. of barley grains were pearled by hand to remove the pericarp and germ. Manganese was determined in the unpearled and in the pearled grains. The unpearled grains contained 20 parts per million of manganese and the pearled grains contained less than 1 part per million, thus showing that practically all of the manganese is contained in the pericarp and germ of the barley grain.

## WHEAT

From the standpoint of the color of the pericarp, wheats may be divided into two general classes, red and white. Red wheats, as the name suggests, have a red or brown pericarp, while white wheats have a light-colored pericarp. Preliminary experiments indicate that a correlation exists between the color of wheats and the amount of manganese which they contain, the red varieties having a larger amount of manganese than the white. A determination of the manganese contained in one sample each of red and white wheat showed that the red variety contained 40 parts per million of manganese, and the white 20 parts per million.

It has been reported that patent flour made from red wheat, although low in the vitamin factors, contains a greater number than does a similar grade of flour made from white wheat. It is also common knowledge that red winter and spring wheats contain a larger percentage of gluten than the white varieties, a fact which prompts the suggestion that manganese may be responsible for this condition.

Samples of whole-wheat grains, wheat bran, and high-grade patent wheat flour were obtained and the manganese in each determined. The following table represents the amount of manganese in parts per million of each of the air-dried materials:

Materials:	Manganese
Wheat grains.....	40
Wheat bran.....	175
Patent flour.....	10

It is apparent that the bran or pericarp contains several times as much manganese as either whole grain or patent flour when equal quantities are considered. It is also clear that the patent flour contains very little manganese, the greater part having been removed in the bran and the germ. The amount of manganese found in patent flour is the same as that found in polished rice. Assuming manganese to be the vitamin factor that is removed in the milling process, we should not expect patent flour to be a preventive of beriberi—an inference which is in accord with the facts as found by other investigators in feeding experiments with animals.

From the foregoing results relative to the manganese content of the whole grains, pericarps, and the highly milled products of barley, wheat, and rice, it is evident that the greater part of the manganese is removed in the bran and polishings; it is therefore logical to assume that a compound of manganese may be the vital factor removed in the milling of these cereals.

## MANGANESE ASSOCIATED WITH VITAMINS IN ANIMAL TISSUES

Various investigations concerning the vitamin content of lean meat generally concur in the conclusion that while lean meat contains some of all the vitamins it is not nearly so rich in these as are some of the glandular organs of the animal body such as the liver, kidneys, spleen, and pancreas. The writer has found no suggestions in the literature as to why these glandular organs are richer in vitamins than the lean muscular meat. Plimmer (9) states that meat consumed in small daily quantities, from 4 to 8 ounces, may not supply enough of any one factor to compensate for an absence of vitamins in the rest of the diet. However, meat consumed in very large quantities protects both from beriberi and scurvy. Plimmer (9) also states that Krogh visited Greenland to study the metabolism of Eskimos and verified the report that they sometimes eat as much as 15 pounds of meat in less than fourteen hours without ill effect. He also says that the chief or only food was boiled seal meat, liver, and blubber. The raw liver of seals and the middle epidermal layers of certain whales are regarded by the Eskimos as a sure protection against scurvy, and their value has been fully confirmed by the medical officers of Greenland. It is also stated by Plimmer that fat or muscle is not as rich in fat-soluble A factor as the fat around the internal organs, e. g., kidney suet.

Having demonstrated that manganese is necessary for the normal metabolic processes in the growth of plants, it is only natural to consider the manganese content of the animal body and whether or not this element is also necessary for the normal metabolic processes in animal life. The Department of Animal Husbandry of the Kentucky Agricultural Experiment Station furnished the organs used in the analysis shown in Table I.

TABLE I.—Amount of manganese found in moisture-free materials

Parts.	Hog.	Sheep.	Steer.
	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>
Brain.....	(a)	3.00	2.56
Heart.....	1.50	2.30	2.50
Lean meat.....	.50	1.25	.80
Fat.....	.00	Trace.	Trace.
Kidney.....	5.00	8.50	6.75
Liver.....	12.50	15.00	14.00
Pancreas.....	(b)	(b)	4.00

<sup>a</sup> Sample insufficient for a determination.

<sup>b</sup> No sample obtained.

From the results shown in the foregoing table it is evident that the liver contains about twice as much manganese as any other part of the animal examined. The kidneys are the part next richest in manganese, while the pancreas, brain, heart, and lean meat follow in order of their manganese content. The fat of each of these animals contained no more than a trace of manganese at the most and the lean meat very little.

The findings here recorded are considerably greater than those reported by Bertrand (1), who has determined manganese in some of the same organs of these and other animals. However, he too finds the largest quantities of manganese in the liver and kidneys. These results for man-

ganese agree very closely with the findings of other investigators in showing that the liver, kidney, heart, brain, and pancreas are richer in vitamin content than other parts of the animal body, thus indicating a close parallelism between vitamin and manganese content in the parts of the animals examined. Undoubtedly manganese has a very important function to perform in the liver, kidneys, and pancreas. Since twice as much manganese was found in the liver as in any other part of the body, it is logical to assume that this accounts for the fact that the liver is also a rich source of vitamins. Several investigators report that they have found the liver of different animals a cure or preventive of the common diseases resulting from the lack of an adequate supply of vitamins.

#### MANGANESE IN THE LIVER OF CODFISH

Investigators have found that the liver of codfish is rich in fat-soluble A vitamin, and many efforts have been made to isolate this vitamin from cod-liver oil, but without success. According to Plimmer (9), fat-soluble A is synthesized in the green parts of plants, and the white leaves of cabbage contain less A factor than the green leaves. Lower plants (marine algae) containing chlorophyll synthesize this vitamin. The marine algae are eaten by small marine animals, which in turn are eaten by larger ones, eventually by codfish. The fat-soluble A factor in the codfish is chiefly concentrated in its liver.

In view of the fact that manganese was found in greater concentration in the livers of the hog, sheep, and cow than in other organs, it is reasonable to expect the liver of the codfish to contain a larger proportion of manganese than any other part of its body. A sample of cod-liver chum, the disintegrated cod-liver tissue after the steaming process, and also some of the clear supernatant cod-liver oil, were obtained from codfish caught near Portland, Me., on May 12, 1923, and each of these materials was examined for manganese. The cod-liver chum was dried at 110° C. and manganese determined in 100 gm. of the moisture-free material. The dry chum contained some oil. Four parts per million of manganese were found in the dry chum, from which it is apparent that the liver of the codfish does contain a considerable quantity of manganese. Two hundred grams of the clear, golden-colored cod-liver oil was burned by means of a wick in a platinum dish and the residue tested for manganese. Only a trace was found.

Another sample of what was claimed to be a high grade of purified Norwegian cod-liver oil which, according to the statement on the label was rich in fat-soluble A vitamin, was examined in a similar way for manganese and approximately one-tenth part per million of manganese was found. The manufacturer from whom the sample was obtained states that the potency of cod-liver oil as a source of fat-soluble A varies with the source of the oil and the treatment it has undergone in the process of refining. It is stated that cod-liver oil that has been subjected to superheated steam or has been highly refined by filtration loses its potency as a source of fat-soluble A factor.

Funk (3) states that he has found the crude cod-liver oil to be richer in fat-soluble A vitamin than the refined oil. He also states that Zilva and Miura (12), in their experiments with animals, found the crude cod-liver oil to have a fat-soluble A potency 250 times greater than butter fat. However, since it has been shown that the liver of the codfish is the richest source of the fat-soluble A vitamin and that the unre-

finer oil is richer in the fat-soluble factor than the refined oil, we can readily account for these facts by assuming that the liver is also richest in manganese; the crude oil would contain less manganese than the liver but more than the refined oil. In the process of refining the crude oil it is filtered through fuller's earth until the oil which was dark in color before filtering is changed to a golden straw color. It is apparent that the fuller's earth removes by adsorption most of the vitamin factor A, which in all probability is a colloidal form of a manganese compound. This assumption is further supported by the fact that Seidell (10) has made use of fuller's earth as a means of separating, by adsorption, some of the vitamin factors. However, he has neither shown nor suggested that it was a compound of manganese that was being adsorbed by the fuller's earth.

#### MANGANESE IN FISH ROE

One pound of fish roe was purchased at a fish market in Lexington, Ky., dried at 110° C., and 100 gm. of the moisture-free material ashed for a manganese determination. Three parts per million of manganese was found in the moisture-free roe of the fish. The livers from freshwater fish, newlights, were obtained from the same market, and 3.75 parts per million of manganese was found in the dry material of the fish livers, which is considerably less than the amount found in the livers of domestic animals. Other investigators state that the flesh of fish is poor in vitamins, whereas their roes and livers are rich in these factors, a fact in harmony with the findings of the author in regard to manganese content.

#### MANGANESE IN MILK

Some investigators state that milk is richest in vitamins at the beginning of the lactation and that its vitamin potency diminishes as lactation progresses. To determine whether the manganese content changes in a similar way, samples were obtained from the first colostrum of a normal cow of the station herd, taken before the calf had sucked, and from the milk of the same cow a month later. Each sample was analyzed by evaporating 1,000 gm. to dryness, ashing and determining manganese in the ash. The results are as follows:

	Colostrum, Apr. 17, 1923.	Milk, May 17, 1923.
Percentage of ash.....	1.154	0.711
Mn. in the ash, parts per million.....	20	4
Mn. in the milk, parts per million.....	.2	.03

The falling off in manganese content is very marked. If manganese is responsible for vitamin potency, the decline of the latter should be in a similar degree. These findings suggest that manganese is mobilized during the time the cow is not producing milk, presumably for the purpose of supplying an element that has important functions to perform during a critical period in the life of the young offspring.

#### MANGANESE IN THE YOLK OF EGGS

Another source of the fat-soluble A vitamin is the yolk of eggs; the white of the egg has proved to be devoid of vitamins.

One dozen fresh, viable eggs were obtained from the poultry farm of the station and hard boiled in distilled water. The eggs were then separated into three parts, shell, whites, and yolks. The whites and

yolks were dried to a constant weight at 110° C., ashed, and the manganese determined in each. The yolks contained 2.72 parts per million of manganese in the moisture-free material, and the whites did not contain a trace of manganese. This fact illustrates the association of manganese with the vitamins and further supports the idea that a compound of manganese is perhaps the vital factor in the yolk of the egg from the standpoint of foods, and also a necessary factor in the development of the embryo contained in the egg.

#### MANGANESE IN TOMATOES

It has been found that tomatoes are rich in vitamins. Four hundred grams of ripe tomatoes were dried to a constant weight at 110° C., ashed, and the manganese determined. In the moisture-free material 12.6 parts of manganese per million were found, corresponding to 0.62 parts per million in the tomatoes used. From the standpoint of manganese content, ripe tomatoes should have a vitamin potency three times as great as that of raw milk, which is in harmony with the results obtained with fresh tomato juice, namely, that it has a high potency as a source of vitamins.

#### MANGANESE IN ORANGES AND LEMONS

It has been shown that the juice of oranges and lemons contains the antiscorbutic vitamin. Cooper (2) has shown that the peel of oranges and lemons also contains the fat-soluble vitamin, and Osborne and Mendel (8) have demonstrated the presence of water-soluble B in the juice of oranges.

Oranges and lemons were examined for their manganese content. The juice from 6 oranges and 12 lemons was filtered separately through cheese cloth and dried to a constant weight at 110° C., ashed, and the manganese determined. The peelings were also dried at 110° C., ashed, and the manganese determined. The results obtained for manganese, in parts per million of the moisture-free material, are as follows:

	Orange.	Lemon.
Juice.....	1.41	3.38
Peel.....	3.20	4.12

From the foregoing results it appears that the lemon contains more manganese in its juice and peel than the orange. However, most investigators assign equal values to each as a source of the antiscorbutic vitamin. The parallelism existing between manganese and vitamins occurs in oranges and lemons.

#### SUMMARY

Small amounts of manganese are widely distributed in nature, and it undoubtedly performs important catalytic functions in plant and animal metabolism. The author has obtained data which show conclusively that manganese is an essential element in plant economy and performs an important function, perhaps catalytic, in the synthesis of chlorophyll. The pericarp and germ of rice, barley, and wheat contain considerable manganese, but the greater part of this element is removed in the polishing and milling processes when these cereals are prepared as highly milled products for food. The value of manganese in the diet has received little consideration heretofore. It is quite logical to assume that manganese is in some way connected with the vital factor removed in highly milled rice, barley, and wheat.

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One pound of fish roe was purchased at a fish market in Lexington, Ky., dried at 110° C., and 100 gm. of the moisture-free material ashed for a manganese determination. Three parts per million of manganese was found in the moisture-free roe of the fish. The livers from freshwater fish, newlights, were obtained from the same market, and 3.75 parts per million of manganese was found in the dry material of the fish livers, which is considerably less than the amount found in the livers of domestic animals. Other investigators state that the flesh of fish is poor in vitamins, whereas their roes and livers are rich in these factors, a fact in harmony with the findings of the author in regard to manganese content.

#### MANGANESE IN MILK

Some investigators state that milk is richest in vitamins at the beginning of the lactation and that its vitamin potency diminishes as lactation progresses. To determine whether the manganese content changes in a similar way, samples were obtained from the first colostrum of a normal cow of the station herd, taken before the calf had sucked, and from the milk of the same cow a month later. Each sample was analyzed by evaporating 1,000 gm. to dryness, ashing and determining manganese in the ash. The results are as follows:

	Colostrum, Apr. 17, 1923.	Milk, May 17, 1923.
Percentage of ash.....	1.154	0.711
Mn. in the ash, parts per million.....	20	4
Mn. in the milk, parts per million.....	.2	.03

The falling off in manganese content is very marked. If manganese is responsible for vitamin potency, the decline of the latter should be in a similar degree. These findings suggest that manganese is mobilized during the time the cow is not producing milk, presumably for the purpose of supplying an element that has important functions to perform during a critical period in the life of the young offspring.

#### MANGANESE IN THE YOLK OF EGGS

Another source of the fat-soluble A vitamin is the yolk of eggs; the white of the egg has proved to be devoid of vitamins.

One dozen fresh, viable eggs were obtained from the poultry farm of the station and hard boiled in distilled water. The eggs were then separated into three parts, shell, whites, and yolks. The whites and



yolks were dried to a constant weight at 110° C., ashed, and the manganese determined in each. The yolks contained 2.72 parts per million of manganese in the moisture-free material, and the whites did not contain a trace of manganese. This fact illustrates the association of manganese with the vitamins and further supports the idea that a compound of manganese is perhaps the vital factor in the yolk of the egg from the standpoint of foods, and also a necessary factor in the development of the embryo contained in the egg.

#### MANGANESE IN TOMATOES

It has been found that tomatoes are rich in vitamins. Four hundred grams of ripe tomatoes were dried to a constant weight at 110° C., ashed, and the manganese determined. In the moisture-free material 12.6 parts of manganese per million were found, corresponding to 0.62 parts per million in the tomatoes used. From the standpoint of manganese content, ripe tomatoes should have a vitamin potency three times as great as that of raw milk, which is in harmony with the results obtained with fresh tomato juice, namely, that it has a high potency as a source of vitamins.

#### MANGANESE IN ORANGES AND LEMONS

It has been shown that the juice of oranges and lemons contains the antiscorbutic vitamin. Cooper (2) has shown that the peel of oranges and lemons also contains the fat-soluble vitamin, and Osborne and Mendel (8) have demonstrated the presence of water-soluble B in the juice of oranges.

Oranges and lemons were examined for their manganese content. The juice from 6 oranges and 12 lemons was filtered separately through cheese cloth and dried to a constant weight at 110° C., ashed, and the manganese determined. The peelings were also dried at 110° C., ashed, and the manganese determined. The results obtained for manganese, in parts per million of the moisture-free material, are as follows:

	Orange.	Lemon.
Juice.....	1.41	3.38
Peel.....	3.20	4.12

From the foregoing results it appears that the lemon contains more manganese in its juice and peel than the orange. However, most investigators assign equal values to each as a source of the antiscorbutic vitamin. The parallelism existing between manganese and vitamins occurs in oranges and lemons.

#### SUMMARY

Small amounts of manganese are widely distributed in nature, and it undoubtedly performs important catalytic functions in plant and animal metabolism. The author has obtained data which show conclusively that manganese is an essential element in plant economy and performs an important function, perhaps catalytic, in the synthesis of chlorophyll. The pericarp and germ of rice, barley, and wheat contain considerable manganese, but the greater part of this element is removed in the polishing and milling processes when these cereals are prepared as highly milled products for food. The value of manganese in the diet has received little consideration heretofore. It is quite logical to assume that manganese is in some way connected with the vital factor removed in highly milled rice, barley, and wheat.

Manganese occurs in largest quantities in the liver, kidney, and pancreas of animals, which leads to the assumption that it has important functions to perform in each of these organs. The occurrence of manganese in those parts of plant and animal tissues with vitamin potency suggests the idea that manganese is either directly or indirectly connected with the vital factors in these tissues.

The value of certain compounds of manganese in medicine has long been recognized, and especially so in preparations intended as cures for nervousness. Feeding experiments are now in progress to test the hypothesis that manganese is a vital factor in animal nutrition.

The fact that manganese is a necessary element in plant growth and that it is found in largest quantities in plant and animal tissues which contain the greatest vitamin potency, leads the author to assume that a relationship exists between this element and the vital factors contained in these tissues.

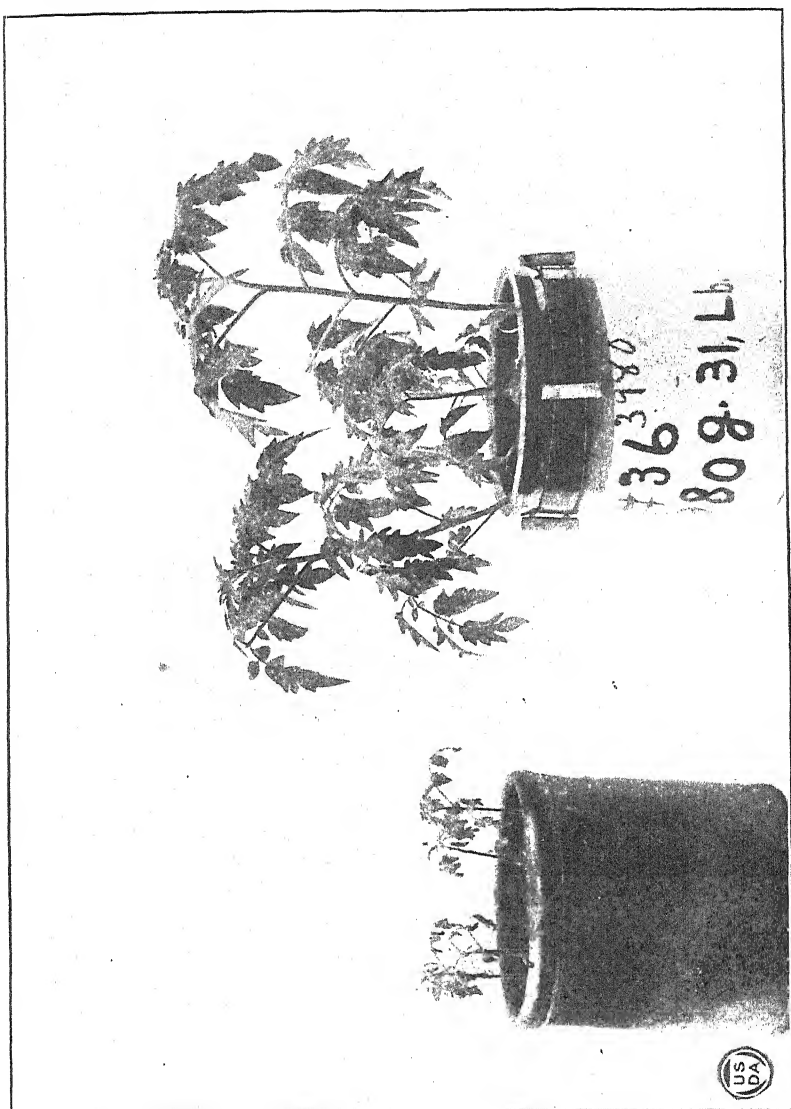
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#### PLATE 1

All plants shown in this photograph are of the same age. The difference in size of the plants in the two pots is due to the fact that manganese was carefully excluded from the mineral nutrients and sand in the pot on the left, whereas a small quantity of a manganese salt was added to the sand in the pot on the right.





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## EXPERIMENTS WITH FLAG SMUT OF WHEAT AND THE CAUSAL FUNGUS, *UROCYSTIS TRITICI* KCKE.<sup>1</sup>

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### INTRODUCTION

*Urocystis tritici* Kcke., the causative organism of flag smut of wheat, though long known to be common in Australia and certain Asiatic countries, was not observed in the United States until May, 1919, when it was discovered by Dr. J. G. Dickson in a wheat field near Granite City, Ill. Because of its reported destructiveness to the wheat crops in other parts of the world, especially Australia, experiments in varietal resistance of wheats were begun in the fall of that year at Granite City. This line of investigation was considered the most expedient at that time because it was known that infestation of soil rather than of seed was the most important factor in the annual occurrence of the disease in Australia. From previous experiments in that country, it had been shown that seed treatment was ineffective in controlling flag smut in infested fields because of infection by viable spores in the soil. Thus, by selecting and sowing varieties which do not become infected in the field, even with artificial inoculation, the disease could be controlled. The results of these and later field experiments relating to varietal resistance and seed treatments have been published recently by Tisdale, Dungan, and Leighty (15).<sup>3</sup>

The data here presented are based on greenhouse experiments begun in the autumn of 1919 at Arlington Experiment Farm, Rosslyn, Va. In the first year only preliminary experiments were attempted, such as the testing of 25 varieties of wheat for susceptibility to flag smut and making a detailed study of the diseased plants. In the autumn of 1920 the number of varieties was increased, and as the work progressed and numerous problems presented themselves it was decided to study some of the physiologic aspects of the disease during the following years. The varietal resistance studies, therefore, cover a period of three years, while the other data are based mainly on a year's investigation conducted at Washington, D. C., and Rosslyn, Va., and a second year's study conducted cooperatively with the Missouri Botanical Garden, St. Louis, Mo.

<sup>1</sup> Received for publication Nov. 23, 1923.

<sup>2</sup> The writer is indebted to Dr. G. M. Reed, formerly pathologist in charge of cereal smut investigations, under whose direction the varietal resistance experiments were begun; to Dr. W. H. Tisdale, who succeeded Doctor Reed in 1920 and whose aid has been invaluable in the greater part of these investigations; and to Dr. B. M. Duggar, of the Missouri Botanical Garden, St. Louis, Mo., who gave helpful advice concerning the 1922-23 experiments reported in this paper.

Most of the seed used in these experiments was obtained from the agronomic division of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, though that of one or two varieties was obtained from each of the State agricultural experiment stations at Knoxville, Tenn., Urbana, Ill., Manhattan, Kans., Davis, Calif., and Pullman, Wash.

<sup>3</sup> Reference is made by number (italic) to "Literature cited", p. 448-449.

## DISTRIBUTION AND ECONOMIC IMPORTANCE

To date (1923) flag smut of wheat has been reported as occurring in the United States, Australia, Japan, China, India, South Africa, Italy, and Spain. In the United States it has been found in Monroe, St. Clair, Madison, Jersey, Macoupin, Greene, Scott, Hancock, and Logan Counties in Illinois; in St. Louis, St. Charles, Warren, Platte, and Buchanan Counties in Missouri; and in Atchison, Leavenworth, Wyandotte, and Miami Counties in Kansas. Field surveys to determine the distribution of flag smut have been made by the State of Illinois and the United States Department of Agriculture each summer for the period from 1919 to 1923. The survey data for each year show that flag smut was found more widely distributed than in the previous year. This increase in the infested area may be due partly to the natural spread of the disease, and partly to the fact that larger areas were surveyed each year, or to either cause alone. From the earlier data it appeared that the disease was spreading rapidly, but evidence from the 1922 and 1923 surveys indicates that it probably has been present for two to several years even in areas reported for the first time to be infested.

During the five years that flag smut has been known to occur in the United States the annual loss due to this disease in the surveyed areas probably has been less than 2 per cent. In parts of some fields, however, 50 per cent of all plants within a given area have been infected. The low average percentage may be due partly to climatic conditions and partly to the fact that most of the time the infested areas have been under State quarantine regulations with regard to disinfection of all grain and threshing machines, the movement of straw, and the sowing of varieties which were found to be fairly resistant to flag smut.

In the literature from Australia, where flag smut was noted first in 1868 (9), losses of from 10 to 50 per cent of the wheat crop, due to this disease, commonly have been reported. However, according to information given by R. J. Noble, of Sydney, Australia, the average annual loss due to flag smut alone is about 3 per cent. In some years it is considerably higher, while in others it is practically negligible. Although flag smut has been distributed widely in the eastern wheat-growing area of Australia, it was not known to occur in Western Australia. However, W. M. Carne, who is engaged in plant research in Western Australia, in a letter to Dr. R. J. Haskell, stated that flag smut was recorded as occurring for the first time in that State in October, 1922, but on one farm only.

Butler (2, *p.* 171-173, *fig.* 56) states that flag smut is confined to the Punjab in India, and that "it has not been reported as causing much damage" in that country.

Hori (5) states that flag smut has been known in Japan since 1895 and that within an area of one-fourth acre, in the Province of Kai, in 1898, 100 per cent of the plants were destroyed before flowering time. It has been reported by Hori (4)<sup>4</sup> from Gumma and Yamanshi prefectures, and from Higo Province by Yoshino (17).<sup>4</sup> Flag smut of wheat is not known to occur in Formosa and Korea. Hori (6)<sup>4</sup> further states that this smut is not so well known to the farmers as the other grain smuts, though infrequently it causes considerable damage.

<sup>4</sup> Japanese original translated by Dr. T. Tanaka, formerly botanical assistant and translator, Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.



Miyake (10) reports the occurrence of flag smut in China and states that it is "very common in Peking and its vicinity."

Putterill (12) reports the occurrence of flag smut in South Africa, and states that it probably has been present there for a "number of years," and that "while the total loss up to now may not be considered very great in that district [Marico], yet in some wheat fields lately visited almost half of the crop was found to be affected."

Flag smut of wheat was collected by E. C. Stakman in 1922 at Rieti, Italy, and at Zaragossa, Spain. The economic importance in either country was not determined.

#### SYMPTOMS OF THE DISEASE

The first indication that a plant in the field is infected with flag smut is the appearance of long grayish-black stripes running parallel with the veins on the upper leaves. At this stage diseased plants in the field appear to be as vigorous as the healthy ones. At a later stage the leaves and upper part of the culms become twisted and curled. Upon close examination of infected plants grown in the greenhouse and field it was found that the young upper leaves showed stripes which were white at their base, becoming whitish gray and finally a lead-gray in color toward the apex of the leaf. Later the epidermis of the leaf ruptures along these stripes and exposes the spores of the fungus in black sooty masses. Plate 1, A, shows some infected leaves with the typical sori. An enlarged portion of an infected leaf also is illustrated (Pl. 1, B). The stripes, or sori, generally are confined to the leaves, although they may occur frequently on the leaf sheaths (Pl. 1, C), occasionally on the upper part of the culm, and still more rarely on the lower glumes and rachis of the wheat head (Pl. 1, D). Plate 1, C, shows the infection occurring on the lower glumes of an underdeveloped head. In many cases all the culms of a diseased plant are infected, although an occasional plant in the greenhouse or field may be found with but one infected culm and the others apparently free from the disease and producing well-filled heads.

In general, the disease seems to stunt the growth of the culms so that no heads are produced. However, in the field in 1922 and 1923 cases were noted where the smutted culms apparently were producing normal heads. These, upon examination, were found in many cases to be sterile, though occasionally a few seeds in each head matured. McAlpine (9) has noted cases where small amounts of shriveled grain were produced from infected culms. In a few of these individuals producing sterile heads it was noticed that the infection was confined entirely to the upper part of the culms, there being no infection of any of the leaves of that culm or of the remainder of the plant.

In the greenhouse, plants of some varieties of spring wheat become infected before they begin to tiller. Very susceptible winter varieties also become infected before or at about the time they begin to tiller. Infection may appear at any time from these young stages of development until the plants are mature.

The culms of infected plants grown in the greenhouse do not tend to curl and twist, as commonly is the case in the field. Infected plants were not allowed to mature, which fact might account for the difference in the effect on plants grown in the greenhouse and on those in the field. It was noticed, however, that in certain varieties, such as Hard Federa-

tion, White Federation, and Bobs, of spring habit, the culms often show this character whether inoculated or not. Obviously, then, this can not be regarded as a trustworthy indication of the disease in these particular cases.

During the first year a detailed record was kept for each infected plant of the different varieties. Each plant was examined separately and the number of well-developed culms determined. In addition, the small undeveloped culms were noted. The nodes from which the roots developed, or the crown, were regarded as No. 1, and the others were numbered successively from the base to the apex. The leaves from the first node usually were dead and dry at the time the plants were examined. This also frequently was true of the leaf at the second node, and sometimes also of that at the third, depending upon the maturity of the plant. However, even in this semidry condition, it was possible to determine whether or not these lower leaves were infected. These plants had reached the heading stage, except in a few varieties where the severity of infection necessitated their removal from the greenhouse before that time.

A summary of the data concerning total culm infection for nine varieties in the crop year 1919-20 is given in Table I.

TABLE I.—Summarized results showing culm infection of wheat plants of different varieties infected with *Urocystis tritici* and grown in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1919-20

Variety.	Source or C. I. No.	Number of plants—		Per-centage of plants with all culms infected.	Culms of partially infected plants.		
		With one to all culms infected.	With all culms infected.		Total number.	Number infected.	Per cent infected.
Baart.....	1697	16	0	0	113	43	38.1
Bobs.....	4990	40	24	60.0	122	93	76.2
Cowra No. 3.....	4119	38	30	78.9	52	37	71.2
Defiance.....	Calif.	38	5	13.2	268	146	54.5
Fultz.....	3598	18	0	0	217	114	52.5
Hard Federation.....	4733	37	29	78.4	51	37	72.5
Harvest Queen.....	5957	41	28	68.3	142	110	77.5
Do.....	Ill.	31	17	54.8	143	116	81.1
Propo.....	1970	18	2	11.1	96	31	32.3
White Federation.....	4981	40	29	72.5	45	29	64.4

In no variety were all culms infected on all infected plants, though in five varieties over 60 per cent of the plants had all their culms infected, while in two varieties no infected plant had all its culms infected. Of the partially infected plants—namely, those which had both sound and infected culms on the same plant—the percentage of culms infected ranged from 32.3 per cent to 81.1 per cent. In eight of these varieties over 50 per cent of the culms were infected.

In general, every well-developed culm of an infected plant showed infection, the severity being indicated by the number, length, and size of the sori. Rarely a leaf showed no infection, though sori appeared on the leaves above and below. As a rule, however, any culm with the second or third leaf infected would show sori on all other leaves above.

Over 1,600 culms were examined to determine the frequency of the occurrence of sori on the lower leaves of the culms. Sori were found on all the leaves on 16.7 per cent of the infected culms; on the second leaf and all leaves above on 48.7 per cent of the infected culms; on the third leaf and all above on 25.3 per cent; on the fourth and those above on 6.7 per cent; on the fifth and those above on 2.4 per cent; and on the sixth and any above on 0.2 per cent. When infection was noted on the first and second leaves there usually was just a trace of the disease, the infection increasing in severity from the third leaf on up to the apical leaf.

The diseased leaves generally were shorter and narrower than the corresponding leaves of healthy culms. The leaves of sound and of infected culms from the same infected plants of 12 varieties were measured and, even in these cases, the diseased leaves were consistently shorter and narrower than the corresponding leaves on the sound culms of the same plant.

#### CAUSAL ORGANISM

Flag smut of wheat is caused by a fungus, *Urocystis tritici* Kcke., belonging to the family Tilletiaceae Tul. It was reported by McAlpine (9) as occurring in Australia as early as 1868 and determined by Wolff (16) in 1873 to be *U. occulta* (Wall.) Rab., the species which occurs on rye (*Secale cereale*). In 1877 Körnicke (7), on examining specimens of flag smut from Australia, separated it from *U. occulta* upon morphological characters alone and named the new species *U. tritici*. However, the name was not adopted, and Saccardo (13, p. 515) gave this name as a possible synonym for *U. occulta*, and Sydow and Butler (14, p. 427), McAlpine (8), and Hori (5) still reported this fungus as *U. occulta*.

McAlpine (9) conducted cross-inoculation experiments with *Urocystis* from wheat and rye in 1907 and 1908. He concluded that "the Flag smut of wheat and rye are not mutually infective, and therefore the name given to Flag smut of wheat by Koernicke in 1877, who received specimens from R. Schomburgk in South Australia, should be retained, viz., *Urocystis tritici*."

*Urocystis tritici* is not known to infect plants of any genus other than *Triticum*, though several genera have been found to be infected with other species of *Urocystis*. In 1921, plants of redtop were received which were infected with *Urocystis*. During the winter of 1921-22 two lots of seed of redtop, Pal timothy, St. John rye (C. I. 130), and Little Club wheat (C. I. 4066) were inoculated, one lot with *U. tritici* and the other with *U. occulta*. These lots of seed were sown on December 1, 1921, in the greenhouse at Rosslyn, Va. There was insufficient spore material from the infected redtop plants to inoculate similar lots of seed. *U. tritici* infected all, or 100 per cent, of the wheat plants, but did not infect any of the rye, timothy, or redtop plants. *U. occulta* infected 7.1 per cent of the rye plants, but did not infect any of the wheat, timothy, or redtop plants.

The spores of flag smut are either solitary or held together in groups of two to several, and the whole completely invested by a layer of small sterile cells (Pl. 2, A). Each spore of a spore ball may germinate, though usually only one or two actually do so (Pl. 2, E, G). However, spore balls with three and even four germinating spores are frequently noted (Pl. 2, H). On germination, the spore sends out a promycelium (Pl. 2, B) with a whorl of one to several sporidia at the apex (Pl. 2, C, D). These sporidia do not become separated from the

promycelium but elongate to form the so-called infection threads (Pl. 2, F a, H d). Frequently not all of the sporidia elongate (Pl. 2, F b), but eventually appear to lose their protoplasm and become hyaline. Fusion of these elongated sporidia and also the production of secondary sporidia (Pl. 2, I a) were observed in a very few cases, though in the great majority of instances they merely became elongated. The size and length of the promycelia, sporidia, and infection threads varied with the conditions of germination.

#### SPORE GERMINATION

The spores of *Urocystis tritici* do not germinate readily or consistently in either distilled or tap water. With spores from material about a month old and kept seven days on soil, McAlpine (9) secured limited germination in a water culture after 24 hours incubation. Approximately 40 per cent of the spores of the previous season germinated after being floated on tap water for about four days.

Various solutions and media were tried under different conditions of moisture and temperature by the writer, but though the spores free from any leaf tissue would germinate under one set of conditions at one time, they would not germinate under apparently similar conditions at another. Thus many negative results were obtained. It may be of interest to note those conditions under which fairly high percentages of germination were secured. In 1920 a method was found which since then has given fairly high and uniform results. In these studies spore-laden leaves were powdered and used as a fine dust-like mixture instead of using spores free from leaf tissue. This material was floated on distilled water in a Syracuse watch glass and placed at 18° to 20° C. Some spores germinated at the end of two and three days, although the best germination usually was had on the fourth day. The germination, once started, proceeded very rapidly, and the elongated sporidia, or infection threads, were quite numerous on the fifth day.

Good germination was secured many times in 1921 in tap-water cultures after two to five days incubation. The spores free from leaf tissue, however, did not germinate readily in distilled water, though a few spores sometimes germinated after four or five days. The optimum temperature for germination was 18° to 20° C., though germination at 25° was recorded in one culture and in certain others at 12° to 13°. Satisfactory germination was obtained at 10° after seven to eight days when spores mixed with pulverized leaf tissue were used. However, in infection experiments herein described no infection was obtained at 25°, though it did occur at 6° to 12°.

In December, 1921, fairly good germination took place after three to four days in juice expressed from wheat seedlings and diluted. In some instances germinating seeds were found to stimulate spore germination. In October, 1921, spores which had been dusted on seeds which were placed on soil in a Petri dish in the laboratory germinated freely after two days, while spores from the same source dusted on soil and on the surface of both tap water and a soil solution, did not germinate after four days of incubation.

During the course of these experiments this stimulating effect of germinating seeds on spore germination was observed also in some tests of the viability of spores buried in the soil. For these experiments 14 samples of spores which had been buried under different conditions were

used. The soil and spore mixture constituting each sample was divided into duplicate portions, each of which was placed in a Syracuse watch glass and 3 cc. of tap water added to each. In one portion of each sample five wheat seeds were placed. After four days there was good spore germination in four of these portions in which seeds were germinating, but there was no germination in the duplicate portions without seeds, with the exception of one in which a single germinating spore was found. Also there were a few spores germinating in one other dish in which no seeds had been placed. After seven days there was good germination in four more cultures containing seeds, but there was no increase in germination in the lots without seeds.

Brown (1) recorded the stimulating effect of volatile substances arising from plant tissues on the germination of spores of *Botrytis cinerea* and other fungi. Plant distillates and various chemical substances also stimulated germination. Noble's data (11) confirm Brown's results on the effect of plant tissues and distillates on spore germination. He found that spores of *Urocystis tritici* "which had been presoaked in water for several days would germinate profusely after the addition of small quantities of wheat seedlings tissue."

#### VIABILITY OF SPORES

In order to test the viability of spores of various ages, seeds of Harvest Queen (C. I. 5957) were inoculated with dry spores of *Urocystis tritici*, collected in different years and at various places, as given in Table II. The inoculations were made by mixing the seed with the spores in small seed envelopes. The inoculum used in this experiment was obtained from leaves which had been dried and kept at ordinary room temperature under laboratory conditions. The inoculated seed was sown in the greenhouse and infection noted on the plants as they matured. The infection percentages are recorded in Table II.

TABLE II.—The effect of age of spores of *Urocystis tritici*, and the locality in which they were produced, on the power to infect Harvest Queen wheat in the greenhouse at Arlington Experiment Farm, in 1921-22

Row number.	Source of spores.	Year collected.	Wheat plants.		
			Total number.	Number infected.	Per cent infected.
37	Australia <sup>a</sup> .....	1919	16	16	100
55	Granite City, Ill. ....	1919	14	9	64.3
46	Rossllyn, Va. ....	1920	17	9	52.9
64, 73	Do. ....	1921	31	21	67.7
10, 19, 28	Granite City, Ill. ....	1921	49	23	46.9

<sup>a</sup> Spores obtained in 1921 from R. J. Noble.

TABLE II shows that the spores produced in 1919 in Australia caused 100 per cent infection, while the others were from 46.9 to 67.7 per cent effective. It may be that the fungus from Australia is more virulent than that obtained in the United States. However, these differences may not be significant, as was shown by the following experiment. Seed of Harvest Queen from the same source as that used in the previous ex-

periment was inoculated with spores collected in the greenhouse at Rosslyn, Va., in 1921, and sown in the same greenhouse bench about 18 days later than the sowings recorded in Table II. Fourteen out of 15 of these plants, or 93.3 per cent, became infected. The seed and spores were from the same source as those used in rows 64 and 73, given in Table II, in which but 67.7 per cent of the plants became infected. Therefore it is reasonable to expect that had the conditions at the time of sowing been similarly varied for all the spore lots used in the inoculations noted in Table II, different percentages of infection might have been obtained. However, the results serve to indicate that the infective power of spores is not destroyed even though they are stored two or three years under laboratory conditions.

In the spring of 1923 germination tests were made with spores from the same collections listed in Table II. In all instances good germination was obtained. Thus it has been shown that the spores collected in Australia and Illinois in 1919 were viable after having been kept for four years in the laboratory.

In order to test the infective power of fresh spores, seed of Harvest Queen was inoculated with spores from infected green leaves collected on January 5, 1922, and sown in clean soil on January 6, 1922, together with one uninoculated row and one row inoculated with dry spores of the previous season for controls. The fresh spores produced an infection of 5.7 per cent and the dry spores 47 per cent, while no infection developed in the uninoculated row. Again in March, 1923, 30 seeds each of Harvest Queen, Hard Federation, and Little Club were inoculated with spores which had been collected the same day, and then were sown in three 10-inch pots containing garden soil autoclaved for 15 minutes at 15 pounds pressure. The pots were set in the soil on a greenhouse bench in order to lessen drying out and to prevent rapid temperature changes. Two plants of Hard Federation, or 6.7 per cent, became infected, but no infection developed in Harvest Queen or Little Club. Thus it was shown that wheat plants can become infected by fresh spores, though the percentages of such infection may be quite small.

#### OVERWINTERING OF SPORES

It having been shown that soil infestation was an important factor in the dissemination of flag smut, and one which made seed treatment partially ineffective, it was thought desirable to secure experimental data on the overwintering of the spores in the soil. On such information recommendations could be based for such practices in soil management as might control the disease.

The following experiment was conducted to determine the ability of spores to overwinter in the soil at Granite City, Ill., in the flag-smut area. Forty-two small cylindrical wire baskets about 1 inch in diameter and 3 inches long were filled with the various mixtures of different soils, diseased leaves, and spores of the previous season, as given in Table III. Besides the native black alluvium of Granite City, there were used sandy loam and basaltic soils from Madison, Wis., and Pullman, Wash., respectively. These baskets were buried in the ground at different depths on October 6, 1921.

One-third of the baskets were dug up on November 1, one-third on November 28, and the final third on April 10, 1922, and the contents sent to the laboratory in small glass vials. A soil suspension was made from each, using about 30 cc. of tap water. The spores in part were

recovered from the soil by fractional centrifuging. The heavier soil particles with many spores were centrifuged out first and then a layer, consisting of many spores and the lighter soil particles, was obtained. To test spore germination, this layer was pipetted off into a Syracuse watch glass, tap water added, and the whole placed in an incubator at 18° C. But it had been found that flag-smut spores often fail to germinate in cultures, although spores from the same source produced abundant infection in susceptible plants inoculated with them. So it was decided to grow seedlings in the heavier residue to determine the viability of these overwintered spores by their infective power. Five seeds of wheat were sown in each soil and spore residuum and allowed to grow in the same chamber with the spore germination tests. Harvest Queen was sown in the series dug up on November 1 and November 28, while in the series dug on April 10, 1922, both Harvest Queen and Bobs were sown.

When these seeds had germinated and each plant had one well-developed leaf, they were transplanted to clean soil in the greenhouse. Infection in the earlier sowings was first observed in January, and the final results were recorded in June. These data, together with the results of the spore germination tests, are recorded in Table III.

TABLE III.—*Viability and infective power of spores of Urocystis tritici buried for varying periods in different types of soil at Granite City, Ill., on October 6, 1921*

Soil and inoculum.	Depth of burial.	Dates baskets were dug from soil.					
		Nov. 1, 1921 (Series 1).		Nov. 28, 1921 (Series 2).		Apr. 10, 1922 (Series 3).	
		Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.	Spore germination.	Infection on Harvest Queen.
	Inches.		Per ct.		Per ct.		Per ct.
Black alluvial soil plus spores.	0-2	—	40.0	+	0	—	0
Do.	2-4	—	0	+	0	—	0
Do.	4-6	—	75.0	—	0	—	0
Do.	6-8	—	75.0	—	0	—	0
Do.	8-10	—	50.0	—	0	—	0
Black alluvial soil plus infected leaves.	0-2	+	0	+	0	—	0
Do.	2-4	—	0	+	0	—	20
Do.	4-6	+	40.0	+	0	—	0
Do.	6-8	+	0	—	0	—	20
Do.	8-10	—	0	+	0	+	0
Basaltic soil plus spores.	4-5	—	33.3	—	0	—	0
Basaltic soil plus leaves.	4-5	—	40.0	+	20	—	0
Sandy loam plus spores.	4-5	+	30.0	—	0	—	0
Sandy loam plus leaves.	4-5	—	0	—	0	—	0

The foregoing results seem to indicate that the infective power of the spores was markedly decreased during the time between the removal of the first and second series, or between November 1 and 28. The spore germinations show a slight increase, due probably to a difference in the method of germination. The two cases of infection obtained in Series 3 from spores buried over five months were noted on Bobs, a very susceptible spring wheat which had not been used for the earlier tests.

Another series of experiments on the overwintering of spores was conducted cooperatively with the Missouri Botanical Garden during the winter of 1922-23. Diseased leaves had been collected in 1922 from plants grown in the field at Granite City, Ill., and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va. These leaves were ground in a food chopper, mixed with two parts of soil in a Mason jar, and placed in small, 2-inch flowerpots on November 24, 1922. These pots were divided into two lots and buried in the soil so that the tops of one lot were 1 inch below the surface of the ground and those of the other 5 inches below. The height of the pots being 2.5 inches, the spore mixtures in each pot of the first lot were from 1 to 3.5 inches and those in the other from 5 to 7.5 inches beneath the soil surface. The two depths at which the pots were buried are recorded in Table IV as 2 and 6 inches, respectively. In addition, six pots, similarly filled, were watered and kept in the greenhouse for 4, 8, and 12 days. At the end of each of these three periods, two pots were buried, one at each depth recorded above. As the viability of the spores is considered to be decreased during long intervals of wetness, the late sowings of spores which had been watered for 4, 8, and 12 days before burial were intended to simulate to a slight extent the periods of fall rains. Thus, if the spores had been in the soil through late summer and early fall, their viability might have been lessened by the time these experiments were begun. As a check on the viability of the spores a part of the soil and spore mixture was kept in the Mason jar until the first pots were dug on January 4, 1923.

At various intervals of about two weeks, some of the pots in each series were taken into the greenhouse. Seed of Little Club was sown in the pots and allowed to grow. The soil temperatures for the different sowings were not the same, the temperatures for March and April being considerably higher than those for January and February. After the resulting seedlings had produced one or two strong leaves, they were transplanted in clean soil in the benches. The pot buried on November 24, with the spores 2 inches below the surface, had its contents destroyed by mice after it was dug up on January 4 and wheat seed sown. Also the seed sown on January 4 in the pot buried 6 inches below the soil surface on December 6 and brought in on January 4 was destroyed by mice. Additional seed was resown in this pot on January 12, 1923. The infection results are given in Table IV.

From Table IV it seems that watering the spores and keeping them in the greenhouse for 4, 8, and 12 days before they were buried in the soil did not diminish their viability. Indeed, four of the rows showed 100 per cent infection, which was slightly higher than that of the control. The spores showed a marked decrease in viability about February 3. The coldest weather of the year was during the two weeks following this time, the minimum temperature recorded being about 1° F. By February 18, the ground had begun to thaw and the pot at the 2-inch depth was brought in, though the one at the 6-inch depth could not be dug from the ground until February 22. There was an infection of 11.1 per cent in the plants grown in the pot buried at the 6-inch depth and brought in on March 16. There was no infection in the plants sown in pots removed from the soil on April 14, but the plants did not grow well because of the high air and soil temperatures in the greenhouse. However, as previously stated, there was infection in the plants sown in April, 1922, in the infested soil which had been removed from the field at Granite City, Ill., on April 10, 1922.



TABLE IV.—Infections of Little Club wheat produced by spores of *Urocystis tritici* buried at different depths and for varying periods during the winter of 1922-23 at the Missouri Botanical Garden, St. Louis, Mo.<sup>a</sup>

Average depth spores were buried.	Date when spores were—		Total number of plants.	Infected plants.	
	Buried.	Exhumed.		Number.	Per cent.
<i>Inches.</i>					
Control			22	21	95.5
2	Nov. 28	Jan. 4	28	25	89.3
6	...do....	...do....	25	25	100
2	Dec. 2	...do....	19	19	100
6	...do....	...do....	27	27	100
2	Dec. 6	...do....	8	8	100
6	...do....	...do....	24	20	83.3
6	Nov. 24	...do....	25	24	96.0
2	...do....	Feb. 3	24	6	25.0
6	...do....	...do....	23	12	52.2
2	...do....	Feb. 18	26	5	19.2
6	...do....	Feb. 22	22	6	27.3
2	...do....	Mar. 1	18	3	16.7
6	...do....	...do....	20	0	0
2	...do....	Mar. 16	18	0	0
6	...do....	...do....	18	2	11.1
2	...do....	Apr. 14	18	0	0
6	...do....	...do....	18	0	0

<sup>a</sup> Experiments in cooperation with the Missouri Botanical Garden.

On October 5, 1921, a plot of land about 10 feet square, near the experimental field at Granite City, Ill., was inoculated thoroughly with flag-smut spores by spading under heavily infected wheat straw. On October 12, 1922, over one year later, clean seed of Harvest Queen wheat was sown in this plot. An uninoculated control plot of equal size, located some distance away, also was sown with uninfested seed of the same variety. In May, 1923, there were nine infected plants, or 2.4 per cent, in a total of 377 in the inoculated plot. None of the 370 plants in the control plot was infected.

Thus it has been shown that, though there is a decrease in viability, the spores are able to survive a winter and subsequent summer in the flag-smut area near St. Louis, Mo. They not only are capable of germination, but actually can infect wheat plants.

#### INFECTION

Regarding the infection of wheat by *Urocystis tritici*, McAlpine (9) states: "Infection occurs in the seedling stage, also when the young shoots are being formed, but not when the plant is above ground, . . ." He proved that infection did occur in the seedling stage, but based his conclusions concerning the infection of new shoots as they are formed during tillering, on some data given by Hecke (3). The latter cut back to the collar shoots of sound plants of the perennial rye (*Secale montanum*) in the autumn. He then dusted spores of *Urocystis occulta* on the exposed collar and covered the plant with manure containing the same kind of spores. In the spring the shoots produced were infected. Discussing Hecke's data, McAlpine says: "But a fresh light has been thrown on the infection of rye, and the same probably applies to wheat. By the experiments of Hecke . . . it was there shown that the spore has not only

the one chance of infecting the primary or terminal bud, but also the numerous chances of infecting the lateral buds produced beneath the surface of the soil and growing out into fresh stalks. There is not only seedling infection, but shoot infection, and it is decidedly to the advantage of the parasite to multiply the points of attack as much as possible."

If it be true that new shoots as well as the plumule are liable to infection before emerging from the soil, then factors which would hasten growth through the soil would shorten the infection period and, therefore, would lessen the chance of infection. Soil moisture and temperature would play an important part by influencing the germination both of the wheat seed and of the smut spores.

Experiments were conducted to determine the relation, if any, between infection and date of sowing, temperature, stage of growth prior to inoculation, and tillering. These experiments are discussed separately. In addition, experiments were conducted to determine the effects of cutting back inoculated plants at different periods of growth on the appearance of flag-smut infection.

#### DATE OF SOWING AS RELATED TO INFECTION

The following experiment was conducted cooperatively with the Missouri Botanical Garden, to determine if any relation existed between the date of sowing inoculated seed and the amount of infection produced. Harvest Queen was used throughout this experiment. Eleven sets of 125 selected seeds each were inoculated and sown in rod rows at three-day or four-day intervals from October 17 to November 21, 1922. The rows were 1.5 feet apart, and the seeds were spaced at intervals of a little less than 2 inches. All inoculations were made simultaneously with dry, ground spore material of the previous season. The vials of inoculated seed for the later sowings were kept in a cool, dry place in order to maintain the viability of both the spores and seed. Infection was first observed on April 28, 1923, on some of the plants of the first sowings. The dates of the various sowings are given in Table V, together with the total number of plants, number of infected plants, and the percentage of infection for each date of sowing.

TABLE V.—Percentages of infection of Harvest Queen wheat by *Urocystis tritici*, as influenced by date of seeding from October 17 to November 21, 1922, in the Missouri Botanical Garden, St. Louis, Mo.<sup>a</sup>

Date sown.	Plants.		
	Total number.	Number infected.	Per cent infected.
Oct. 17.....	31	19	61.3
Oct. 20.....	48	24	50.0
Oct. 24.....	37	14	37.8
Oct. 27.....	26	6	23.1
Oct. 31.....	24	5	20.8
Nov. 3.....	24	7	29.2
Nov. 7.....	50	7	14.0
Nov. 10.....	79	19	24.1
Nov. 14.....	73	0	0
Nov. 17.....	72	0	0
Nov. 21.....	69	0	0

<sup>a</sup> Experiments in cooperation with the Missouri Botanical Garden.

The percentage of infection decreased from 61.3 per cent in the sowing of October 17 to 20.8 per cent in the sowing of October 31. There was no infection in the rows sown on or after November 14. These variations in the percentage of infection were due probably to soil temperature and moisture conditions, which inhibited the germination of the spores at the time the wheat was germinating.

#### TEMPERATURE AS RELATED TO INFECTION

A series of experiments was arranged in order to determine the range of temperature within which actual infection could occur. On December 1, 1921, 40 seeds of each of five susceptible varieties of wheat (Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation) were inoculated heavily with flag-smut spores by moistening the seeds and rolling them in dry spores. The seeds were then incubated between moist blotting papers in the manner prescribed for standard seed-germination tests. Through the kindness of W. L. Goss, of the Seed Testing Laboratory of the Bureau of Plant Industry, germination chambers were secured in which the temperature was controlled within narrow limits by electric thermostats and in which a humidity of practically 100 per cent was maintained. Recording maximum and minimum thermometers were placed in each chamber. The three chambers used had average temperatures of 17.8° C., 22.3°, and 25.8°, with fluctuations between 17° and 19°, 21.5° and 23.5°, and 25° and 26.6°, respectively, during the time the seeds were in the chambers. A fourth lot of seeds of the same varieties was placed in an ice box, the temperature of which ranged from 6° to 12° during the 30 days of the experiment.

When the seedlings in each chamber had reached the stage where the first leaf had pushed through the coleoptile, they were washed thoroughly with the aid of a brush until no spores were visible on the seeds. They were then transplanted to clean soil in the greenhouse, in December, 1921, and allowed to grow until flag-smut lesions appeared or, if the plants did not become infected, until maturity. The infection percentages obtained at each temperature are given in Table VI.

TABLE VI.—The effect of temperature on infection of five varieties of wheat by *Urocystis tritici* when inoculated seed was germinated on blotters in temperature chambers, then washed and transplanted in greenhouse soil in December, 1921

Variety.	Average temperature.							
	9° C.		17.8° C.		22.3° C.		25.8° C.	
	Total number plants.	Infected plants.	Total number plants.	Infected plants.	Total number plants.	Infected plants.	Total number plants.	Infected plants.
	No.	P. ct.	No.	P. ct.	No.	P. ct.	No.	P. ct.
Bobs.....	30	5 16.7	25	3 12.0	19	6 31.6	27	0 0
Hard Federation.....	29	0 0	19	0 0	16	7 43.8	26	0 0
Harvest Queen.....	23	0 0	32	6 18.8	28	9 32.1	30	0 0
Little Club.....	23	0 0	23	1 4.3	27	6 22.2	31	0 0
White Federation.....	.....	.....	18	0 0	14	2 14.3	14	0 0
All varieties.....	105	5 4.8	117	10 8.5	104	30 28.8	128	0 0

The data show that only one variety, Bobs, became infected at 6° to 12° C. and that the highest percentage infection for each variety occurred at 21.5° to 23.5°. The fact that no infection developed in any of the five varieties when grown at 25° to 26.6° is probably due either to the accelerated growth of the wheat plants at this higher temperature, which enabled them to escape infection, or to the inhibition of spore germination, or to both effects.

Even at the optimum temperature all of the percentages of infection are relatively low for such susceptible varieties. In the varietal resistance experiments for 1921-22, herein described, in which the inoculated seed was sown in the soil, the percentages of infection in these same varieties were 86.7 for Bobs, 80.8 for Hard Federation, 88.5 for Harvest Queen, 97.8 for Little Club, and 97.4 for White Federation.

A preliminary experiment on the effect of controlled soil temperatures on infection was conducted by W. H. Tisdale and R. W. Leukel in the greenhouse at Arlington Experiment Farm in the spring of 1923. An equal amount of soil was placed in each of 12 galvanized cans. Two cans were set in water in each of six tanks held at 10°, 15°, 20°, 24°, 28°, and 32° C., respectively. Twenty seeds of Bobs wheat were sown in each can on March 26. The seeds were spaced at regular intervals in a circle, 1.5 inches from the border of the can, and covered with about 1 inch of soil. No smut developed in any of the cans except the ones in which the soil temperature was held at 20°. In these cans 5, or 12.5 per cent, of the 40 plants became infected with flag smut.

#### STAGE OF GROWTH AS RELATED TO INFECTION

In the case of seedling infection it is stated by McAlpine (9) that infection occurs before the plant emerges from the soil. Soon after emergence the coleoptile, or sheath, which completely invests the plumule, is broken, and the latter appears. In order to determine the stages of seedling growth at which infection could occur, the following experiments were conducted. Seed of Bobs, Hard Federation, Harvest Queen, Little Club, and White Federation, the same varieties used in the temperature experiments, were placed between moist blotters, at 18° to 20° C. in a seed-germination chamber on December 6, 1921. When these seeds were at three definite stages of germination—namely, (1) testa, or seed coat, just broken; (2) plumule about 1 to 1.5 cm. long, and three strong roots developed; and (3) coleoptile just broken, those in each stage were divided into two lots. In addition, dry, ungerminated seed was divided into two lots. Both lots were inoculated and placed in clean soil in the greenhouse December 9 to 16, 1921. One lot of each was inoculated by rolling the seed or seedling in dry spores and the other by immersing in a spore suspension. This suspension consisted of spores soaked in tap water for at least two days. The spores in suspension were not necessarily germinating. It was thought that if the time between the transplanting and emergence of the seedlings was very short the dry spores might not have time to germinate and infect the seedlings before the latter came through the ground. On the other hand, if the spores were thoroughly soaked they could germinate in a very short time. Unfortunately, the emergence dates for these seedlings were not recorded, although these data would have been valuable in correlating rate of growth and infection. Table VII contains the results for the two lots of each variety inoculated with the soaked and dry spores, respectively, at the three stages of growth described above.

TABLE VII.—Results of inoculating wheat seedlings at three definite stages of growth with both soaked and dry spores of *Urocystis tritici* and planting them in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., December 9 to 16, 1921

Variety.	Seedlings inoculated with—						Average percent- age of infection including both lots.
	Lot 1. Soaked spores.			Lot 2. Dry spores.			
	Total plants.	Infected plants.		Total plants.	Infected plants.		
SEED COAT JUST BROKEN.							
		<i>Number.</i>	<i>Per cent.</i>		<i>Number.</i>	<i>Per cent.</i>	
Bobs. ....	36	4	11.1	35	17	48.6	29.6
Hard Federation. ....	34	5	14.7	33	16	48.5	31.3
Harvest Queen. ....	32	6	18.8	34	6	17.6	18.2
Little Club. ....	31	20	64.5	28	10	35.7	50.8
White Federation. ....	33	20	60.6	30	23	76.7	68.3
PLUMULE AND THREE ROOTS DEVELOPED.							
Bobs. ....	35	5	14.3	34	12	35.3	24.6
Hard Federation. ....	35	9	25.7	36	11	30.6	28.2
Harvest Queen. ....	34	10	29.4	33	4	12.1	20.9
Little Club. ....	30	25	83.3	31	18	58.1	70.5
White Federation. ....	29	18	62.1	29	16	55.2	58.6
COLEOPTILE BROKEN.							
Bobs. ....	35	0	0	36	0	0	0
Hard Federation. ....	36	0	0	36	0	0	0
Harvest Queen. ....	35	0	0	34	0	0	0
Little Club. ....	32	0	0	33	0	0	0
White Federation. ....							
CONTROL: DRY UNGERMI- NATED SEED.							
Bobs. ....	17	8	47.1				47.1
Hard Federation. ....	17	8	47.1				47.1
Harvest Queen. ....	15	7	46.7	17	9	52.9	50.0
Little Club. ....	8	8	100	8	6	75.0	87.5
White Federation. ....	7	5	71.4	7	5	71.4	71.4

Abundant infection occurred in the plants inoculated at the first two stages, but in no case was there infection of any plant which was inoculated after the coleoptile was broken. The highest percentage of infection in the three-root stage was 83.3 per cent in Little Club, and in the stage where the seed coat was just broken there was 76.7 per cent in White Federation. There apparently was no relation between the condition of the inoculum at time of application and the percentage of infection produced in each case. In some instances where the inoculations were made at the same stage of growth the dry inoculum produced a higher percentage of infection, while in others the spore suspension produced the higher percentage. Four of five varieties had higher percentages of infection when inoculated with dry spores at the time the seed coat was just broken than when the inoculations were made at the three-root stage. All of the varieties inoculated with the spore suspension had higher percentages of infection when they were inoculated at the three-root stage than when the seed coat was just broken. In the varietal-resistance experiments herein described infections of 100 per cent were

recorded in a few varieties. Obviously, in these cases, inoculating the seeds with dry spores gave as high a percentage as could have been obtained if the seeds had been inoculated with soaked or even germinating spores. These results would indicate that the period favorable for infection is before the seedling comes through the ground, or before the coleoptile is broken.

In order to test the possibility of infection during the tillering stage, when new shoots are produced from the crown of the plant, the following experiments were conducted: One lot of smut-free seed of Harvest Queen, Little Club, and White Federation, the same varieties used in the foregoing experiments, was sown in clean soil on November 28, 1921. A similar lot was inoculated with dry spores of the previous season and sown on the same day. Three rows of each variety in the two lots were sown. On December 13, when the plants in both lots had developed one strong leaf and a small second one, they were removed from the soil. Only two rows of each variety in the two lots were removed. For a control the third row in each instance was not disturbed. The uninoculated plants then were inoculated by rolling the roots and lower part of the stem in dry spores and were transplanted to the rows in which the inoculated seed had been sown. The inoculated plants were washed free from all soil particles and the old attached seed washed and brushed until all traces of the spores were removed. They then were planted in the clean soil where the uninoculated seed had been sown. Thus, there was an exact reversal of position. Both sets also were inoculated, one on the original seed and the other when the plants were about two weeks old. The results are given in Table VIII.

TABLE VIII.—Infection of three varieties of wheat by *Urocystis tritici* after inoculating seed and seedlings in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., from November 28 to December 13, 1921

Variety.	Control.				Uninoculated seed; seedlings inoculated and transplanted to infested soil.			Inoculated seed; seedlings washed and transplanted to clean soil.		
	Clean seed in clean soil.		Inoculated seed in clean soil.		Uninoculated seed; seedlings inoculated and transplanted to infested soil.				Inoculated seed; seedlings washed and transplanted to clean soil.	
	Total number plants.	Infected plants.	Total number plants.	Infected plants.	Total number plants.	Infected plants.	Total number plants.	Infected plants.	Total number plants.	Infected plants.
Harvest Queen.....	16	No. 0 P. ct. 0	18	No. 14 P. ct. 77.8	30	No. 1 P. ct. 3.3	34	No. 30 P. ct. 88.2	30	No. 18 P. ct. 90.0
White Federation.....	17	0 0	14	11 78.6	30	0 0	20	18 90.0	30	30 93.8
Little Club.....	17	0 0	14	14 100	35	1 2.9	32	30 93.8	30	30 93.8
All varieties.....	50	0 0	46	39 84.8	95	2 2.1	86	78 90.7	86	78 90.7

There was one plant each of Harvest Queen and Little Club infected in the lot which was inoculated when the plants were about two weeks old, but no infection appeared in White Federation. No other infection was noted on plants which were inoculated after the seedlings had emerged. There was 88.2 per cent or more of infection in each variety which was inoculated at the time the seeds were sown. From these results and from the data obtained in the previous experiment where

no infection occurred in 277 plants when inoculation was made after the coleoptile was broken, it would seem that in the greenhouse, at least, shoot infection was almost negligible.

#### RELATION OF CUTTING BACK INOCULATED PLANTS TO DEVELOPMENT OF SMUT

In addition to the possible infection of shoots during the process of tillering, there is another type of shoot infection which evidently comes from within the plant and is the result of the original seedling infection.

In the spring of 1921 inoculated plants of Bunyip, Cedar, and Comeback, grown in the greenhouse at Arlington Experiment Farm, apparently were free from the disease. Each plant had about four culms, all of which were yellow and well headed at the time of note taking. As these varieties had been practically free from the disease in the previous season, and as other infected varieties needed immediate attention, they were allowed to mature. However, the plants were watered each day, and small secondary shoots soon began to develop at the base of the mature culms. On later examination several of these shoots were found to be infected, even though the mature plants had shown no infection. The occurrence of the disease in these small, secondary shoots, while the headed culms of the same plants remained smut-free, possibly may be explained in two different ways. First, it may be assumed, as McAlpine has suggested, that they were infected by spores present in the soil at the time of emergence; or, second, that they were infected from mycelia already in the plant, which, for some reason, had not been able to form spores in the older leaf tissue.

Greenhouse experiments were conducted in 1921-22 to determine the possible source of infection in such secondary shoots and to study the effect of cutting back the plants at various stages of growth on the production of diseased secondary shoots. The results of the experiments in which seedlings had been inoculated immediately following rupture of the coleoptile and later, during tillering, have already been given. There was no infection of any shoot when the inoculations were made just after the coleoptile had been broken. Two plants out of a total of 95 were infected when inoculations were made after the seedlings were about two weeks old.

Again, 50 uninoculated seedlings of Harvest Queen were cut to the level of the ground immediately after the coleoptile became ruptured. The cut surface of each seedling and the soil around it were heavily inoculated with flag-smut spores. No infection developed in any of these plants. In later experiments no new cases of infection have been noted where inoculation of the soil and plants was delayed until the latter had emerged from the soil.

None of the soil in which the cut-back plants were grown was inoculated with flag-smut spores. The spores were sown with the seed and, therefore, were about 2 inches under ground. Thus there were no spores near the surface of the soil excepting those which might have been carried up through the soil by the plumule during its growth.

The crowns of wheat plants grown in the greenhouse were practically on the surface of the soil, so that often the secondary shoots never came in contact with the soil. The shoots arising from the base of the culms often were pressed closely to the culms. In cases of this kind the basal leaf sheaths of the culm incased both the secondary shoots and the lower

part of the culms until the shoots were from 1 to 3 inches high. The secondary shoots arose from the crown or from the first, second, and third nodes above the ground. Infection often was noted on shoots which arose from these points of origin. Plate 3, A, shows a cut-back plant with an infected shoot which arose from the second node, or the first node above ground, of a culm which had been cut. This infected shoot from the second node is enlarged in Plate 3, B.

From the fact that the infected shoots often arise from the nodes above ground, where the chances for infection are very few, and from the difficulty encountered in producing infection where inoculations were made after the plants were above ground, it would seem that the smut appearing on the secondary shoots arose from hyphae already inside the tissues of the host plant.

Seventeen winter varieties and 11 spring varieties were used in experiments to study the effect of cutting back wheat plants on the appearance of infection. These varieties were resistant, slightly susceptible, and, in a few instances, quite susceptible in previous varietal experiments. A susceptible spring and a susceptible winter variety, Bobs and Harvest Queen, were used as controls for infection. Four rows of each variety were sown 6 inches apart, placing 18 inoculated seeds 2 inches apart in each row. The plants in row 1 of each variety were cut back to about 1 inch in height when they had two leaves and were two to three weeks old. The plants of the second row of each variety were cut back to 2 to 3 inches in height when they had two or three small culms, or when infection was showing in the susceptible varieties used as controls. Plants in the third rows were cut back when beginning to head, and those in the fourth rows were left uncut and used as controls on the plants which were cut back. These different series of cut-back plants are hereafter referred to as rows 1, 2, 3, and 4.

The appearance of smut lesions was watched for closely, and all infected plants removed as soon as observed, so that in each instance only the smut-free plants were cut back. Of the 11 spring varieties treated in this manner 3 showed no infection and 2 an infection of 1.6 and 5.4 per cent, respectively, but which did not seem to be influenced by cutting back the plants. However, 5 varieties developed smut in the cut plants either earlier or in a greater percentage than in the plants which were not cut back. Of the 17 winter varieties, 10 showed no smut, while 4 showed only one or two infected plants. Thus in only 3 winter varieties was the development of smut influenced to any extent by cutting back the plants. The percentage of plants infected in each row of the 8 different varieties which became infected is given in Table IX.

There was no uniform plant response from each variety at each stage of development when cut back. In all varieties where the plants were cut back in the two-leaf stage, there was a greater percentage of infection than in the control rows which were not cut back. There was considerable variation in the percentage of infection in rows 2 and 3. Moreover, it was observed that clipping the culms not only influenced the percentage of infection, but, in many instances, the interval between sowing and appearance of smut was considerably shortened.

Clipping the plants after they had begun to head was the most favorable with regard to both the percentage and the appearance of infection in Bunyip. Small, secondary shoots at once began to develop in row 3, and the lesions appeared 17 days earlier in this row than in rows 2 and



4, and 34 days earlier than in row 1. Row 3 had a final infection percentage of 58.3 in comparison with 31.3 per cent in the control row. There was one plant in row 1 which was cut back twice before infection was noted—once in the two-leaf stage, and again after the plant had headed. Finally a small secondary infected shoot was put out from the first node above ground.

TABLE IX.—Percentages of infected plants in eight wheat varieties inoculated with *Urocystis tritici*, and cut back at various stages of growth, in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in 1921-22

Variety.	C. I. No.	Row number and stage when cut back.			Control.
		1 Two-leaf.	2 Time of normal infection.	3 Heading.	4 Not cut.
Bunyip.....	5012	40.0	40.0	58.3	31.3
Comeback.....	4991	90.9	72.7	84.6	" 20.0
Florence.....	5129	50.0	50.0	60.0	31.6
Sonora.....	4293	36.3	23.0	22.2	25.0
Pacific Bluestem.....	3019-3	83.3	43.7	42.8	33.3
Flint.....	6612	54.5	26.6	15.1	41.2
Fultz.....	3598	40.0	77.7	13.3	18.8
Jones Fife.....	5608	64.2	44.4	46.6	42.9

<sup>a</sup> Normal infection. An additional 73.3 per cent of the plants showed infection in small secondary shoots which appeared after the first culms had matured.

A striking difference in infection percentages was shown in the various rows of Comeback. It will be noticed from figure 1 that by the time there was 72.7 per cent of infection in row 1 there was 15.3 per cent in row 3, 9 per cent in row 2, and 20 per cent in row 4. None of the plants in rows 2, 3, and 4 had been cut back at this time. After all infected plants had been removed from the four rows the remaining sound plants in row 2 were cut the same day. Row 3 was cut back about one month later. Small shoots soon were produced at the crown and nodes, and many of these were infected with flag-smut sori. The dotted line in row 4, or the control, represents the infections which appeared in the small secondary shoots which were produced after the culms were practically mature.

Row 1, sown to Florence, produced 18.4 per cent more smut than the control, and the period of smut maturation was reduced by 30 days. Sixty per cent of the plants in row 3 were infected. This was the maximum for this variety, and exceeded by 28.4 per cent the infection recorded for the control row.

The final percentages of infection for Sonora did not show great differences. Row 1 produced 36.3 per cent, or 11.3 per cent more infection than developed in the control row. Row 2 contained 23.0 per cent of infection in comparison with 25.0 per cent in the control, but it should be noted that the total percentage of smut recorded for row 2 had developed 22 days before infection appeared in row 4, and 73 days before the last infected plant was noted in row 4. Although row 1 showed the highest percentage of infection, no lesions appeared in row 1 until 13 days after

TABLE X.—Infection of wheat, spelt, and emmer by *Urocystis tritici* produced by inoculating the seed with dry spores and sowing in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., during one or more of the three years, 1919, 1920, and 1921

Crop and variety.	Source or C. I. No.	1919-20				1920-21				1921-22		Average number of infected plants.		
		Plants.		Culms.		Plants.		Culms.		Plants.		Years averaged.	Plants.	Infected.
		Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infected.	Total.	Infected.			
WINTER WHEAT.														
China.....	180					No.	P. ct.	No.	P. ct.	No.	P. ct.		No.	P. ct.
Fulcaster.....	6612					50	2.0	293	0.3	9	11.1	2	2	3.4
Fulcaster.....	6162	35	0	165	0	60	25.0	605	4.5	17	41.2	2	22	28.0
Fultz.....	3598	40	45.0	364	31.3	44	0	237	0	14	0	3	0	0
Gipsy.....	5579					52	11.5	269	3.7	15	0	2	6	9.0
Goens.....	3428					57	78.9	340	54.4	10	18.8	3	66	58.4
Harvest Queen.....	5957	48	85.4	434	79.5	53	3.3	291	1.0	16	0	2	2	2.9
Do.....	Ill.	41	75.6	420	73.8	59	6.8	366	1.1	17	5.0	2	5	6.6
Honor.....	6161					116	93.1	1,094	87.1	104	88.5	3	241	89.9
Hussar.....	4843											1	31	75.6
Do.....	6553					52	0	290	0	8	12.5	2	1	1.7
Illini Chief.....	5406									46	0	1	0	0
Jones Fife.....	5608									29	0	1	0	0
Kanred.....	5146	37	5.4	217	.9	58	0	348	0	14	0	2	0	0
Martin.....	1974					45	86.7	284	75.7	14	42.9	2	45	76.3
Do.....	4463					54	0	369	0			2	2	2.2
Mealy.....	5824	45	2.2	196	1.0	61	9.8	350	2.0	12	0	2	6	8.2
P-1066.....	5879									60	0	1	0	0
P-1068.....	5880									15	13.3	3	12	10.6
Pocle.....	3489	46	0	189	0	53	17.0	273	.7	6	0	1	0	0
Purplestraw.....	1915									15	0	1	0	0
Red May.....	5596	39	0	229	0	62	66.1	425	42.6	14	0	3	0	0
Red Rock.....	5976	43	0	222	0	45	0	242	0	13	0	3	0	0
Red Wave.....	5582					50	0	267	0	13	0	3	0	0
Treadwell.....	3527					48	72.9	313	36.1			1	35	72.9
Turkey.....	5966					45	0	419	0	10	0	2	0	0
						58	0	485	0	17	0	2	0	0
SPRING WHEAT.														
Baart.....	1697	45	35.6	251	17.1	55	29.1	219	9.1			2	32	32.0
Bobs.....	4990	43	93.0	257	84.0	124	98.4	541	94.5	30	86.7	3	188	95.4
Bunyip.....	5012	49	2.0	158	.6	59	5.1	177	1.7	16	31.3	3	9	7.3
Cedar.....	4117	52	0	210	0	60	6.7	221	2.3	14	28.6	3	8	2.6
Chul.....	2227					62	0	258	0	14	14.3	2	2	2
Comeback.....	4991	48	0	211	0	64	43.8	233	14.8	15	93.3	3	42	33.1
Cowra No. 3.....	4119	41	92.7	264	89.8	47	87.2	370	81.4	31	0	3	79	66.4
Defiance.....	Calif.	43	88.4	317	52.7	61	24.6	242	9.5			2	53	51.0
Dicklow.....	3663					40	50.0	176	30.1			1	20	50.0
Early Defiance.....	6480	43	0	183	0	55	0	227	0	17	0	3	0	0
Florence.....	5129	44	0	157	0	46	13.0	187	3.7	19	31.6	3	12	11.0
Galgals.....	2398	47	0	264	0	60	0	342	0	16	0	3	0	0
Hard Federation.....	4733	51	72.5	273	68.1	56	98.2	230	95.2	26	80.8	3	113	85.0
Little Club.....	4066	50	2.0	235	.9	56	100	324	95.7	45	97.8	3	101	66.9
Marquis.....	3641	51	7.8	292	2.4	63	3.2	270	1.1	15	0	3	6	4.7
Pacific Blue-stem.....	3019-3	48	4.2	206	1.5	61	4.9	275	1.8	12	33.3	3	9	7.4
Peliss.....	1884					58	0	169	0	13	0	2	0	0
Prope.....	1970	51	35.3	247	16.6	64	62.5	322	51.9			2	58	50.4
Sonora.....	4293	51	2.0	185	.5	57	29.8	272	8.5	16	25.0	3	22	17.7
White Federation.....	4981	45	88.9	207	84.5	50	100	247	92.7	39	97.4	3	128	95.5
EMMER.														
Black Winter.....	2337	40	0	108	0							1	0	0
SPELT.														
Alstrom.....	1773	30	10.0	197	2.0							1	3	10.0

The percentage of infection for the same strains of many varieties was found to be much higher when grown in the greenhouse than when grown

in the field. Tisdale, Dungan, and Leighty (15) give the average percentage of infection for the different varieties grown in the field for a number of years. The same strains of some of the varieties grown by them in the field also were grown in the greenhouse by the writer. The average percentages of infected plants grown under both field and greenhouse conditions are given in Table XI.

TABLE XI.—Percentage of infection of the same strains of wheat, spelt and emmer inoculated with *Urocystis tritici* and grown in the field at Granite City, Ill., and in the greenhouse at Arlington Experiment Farm, Rosslyn, Va., in the same three years, 1919, 1920, and 1921

Variety.	Source or C. I. No.	Field.		Greenhouse.	
		Average infection.	Number of years grown.	Average infection.	Number of years grown.
WHEAT.					
China.....	180	<i>Per cent.</i> 1.6	2	<i>Per cent.</i> 3.4	2
Dawson.....	6161	5.8	2	1.7	2
Fulcaster.....	6162	1.2	3	0	3
Fultz.....	3598	2.5	2	58.4	3
Gipsy.....	5579	.7	3	2.9	2
Goens.....	3428	2.5	2	6.6	2
Harvest Queen.....	5957	24.9	3	89.9	3
Do.....	Ill.	18.1	1	75.6	1
Hussar.....	4843	0	1	0	1
Illini Chief.....	5406	3.0	2	0	2
Jones Fife.....	5608	4.8	2	76.3	2
Kanred.....	5146	2.5	3	2.2	2
Martin.....	Wn. 1092	0	1	0	1
Mealy.....	5824	7.3	2	10.6	3
P-1066.....	5879	.3	2	0	1
P-1068.....	5880	0	2	0	1
Poole.....	3489	Trace.	3	0	3
Purplestraw.....	1915	9.7	2	66.1	1
Red May.....	5596	1.0	2	0	3
Red Rock.....	5976	.2	3	0	3
Red Wave.....	5582	Trace.	1	72.9	1
Treadwell.....	3527	2.8	3	0	2
SPELT.					
Alstrom.....	1773	0	2	10.0	1
EMMER.					
Black Winter.....	2337	0	2	0	1

Many of the varieties which were free from infection when grown in the greenhouse were reported as being slightly susceptible in the field. On the other hand, one of the most susceptible varieties, Harvest Queen (Red Cross), had a three-year average infection of 24.9 per cent in the field. The same strain of Harvest Queen grown in the greenhouse during the same three years had an average infection of 89.9 per cent, or a difference of 65 per cent. Other varieties which showed great differences in percentages of infection were Fultz, Jones Fife, Purplestraw, and Red Wave. The fact that the area quarantined for flag smut is located in the winter-wheat section has precluded any experiments on varietal resistance of spring wheat in the field.

## SUMMARY

Flag smut of wheat occurs in the United States, Australia, Japan, China, South Africa, Italy, and Spain. Although heavy losses have been reported from other countries, it has been estimated that in the five years that flag smut has been known in the United States there has been less than 2 per cent of loss in the infested areas.

Flag smut of wheat is caused by *Urocystis tritici* Kcke., which produces sori on the leaves, stalks, and glumes. Badly infected plants do not head. The spores do not germinate readily in tap or distilled water, and special conditions are necessary for uniform germination. Spores kept under laboratory conditions were viable for at least four years. They are able to live through the winter in the soil in the vicinity of St. Louis, Mo. These overwintered spores are capable not only of germinating but of infecting wheat plants.

Sowing inoculated wheat at successive dates in the fall at St. Louis, Mo., resulted in a general decrease in the percentages of infection. There was no infection in the sowing of November 14 or in later sowings.

Infection occurred at 6° to 12° C., 17° to 19°, and 21.5° to 23.5°, but did not occur at 25° to 26.6°. The highest percentage of infection occurred at 21.5° to 23.5°. The most favorable stage of growth for infection was in the seedling stage before the coleoptile was broken and before the seedlings emerged from the soil.

Infection occurred either earlier or in a greater percentage in a few varieties which had been inoculated and cut back at different stages of growth than in the same varieties which had not been cut back.

Fulcaster, Poole, Red May, Red Rock, Early Defiance, and Galgalos remained smut-free during the three years of this experiment; Illini Chief, Treadwell, Turkey, and Peliss, grown for only two years, also were smut-free. The percentages of infection of susceptible varieties grown in the greenhouse were considerably higher than those of the same strains grown in the field.

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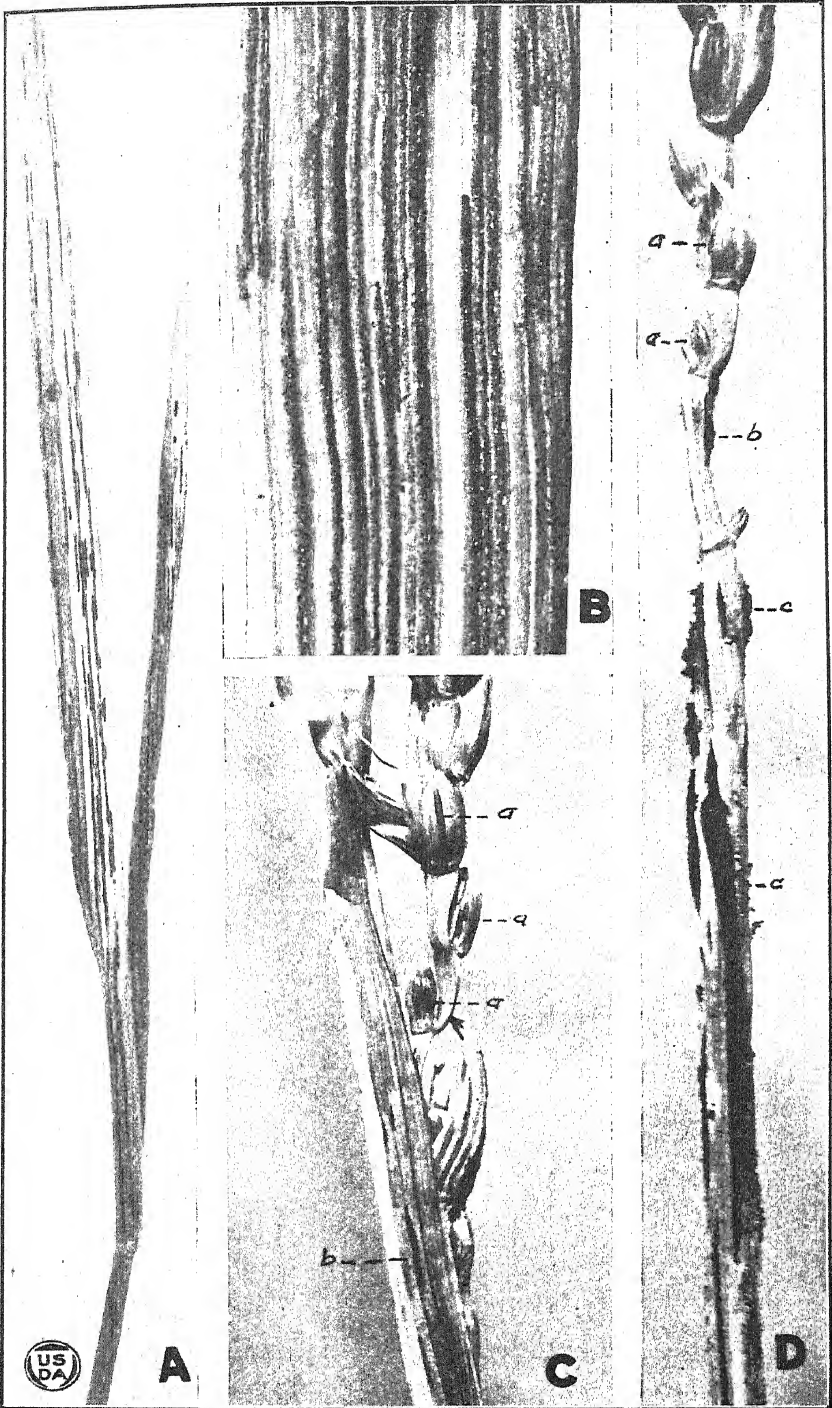
PLATE I

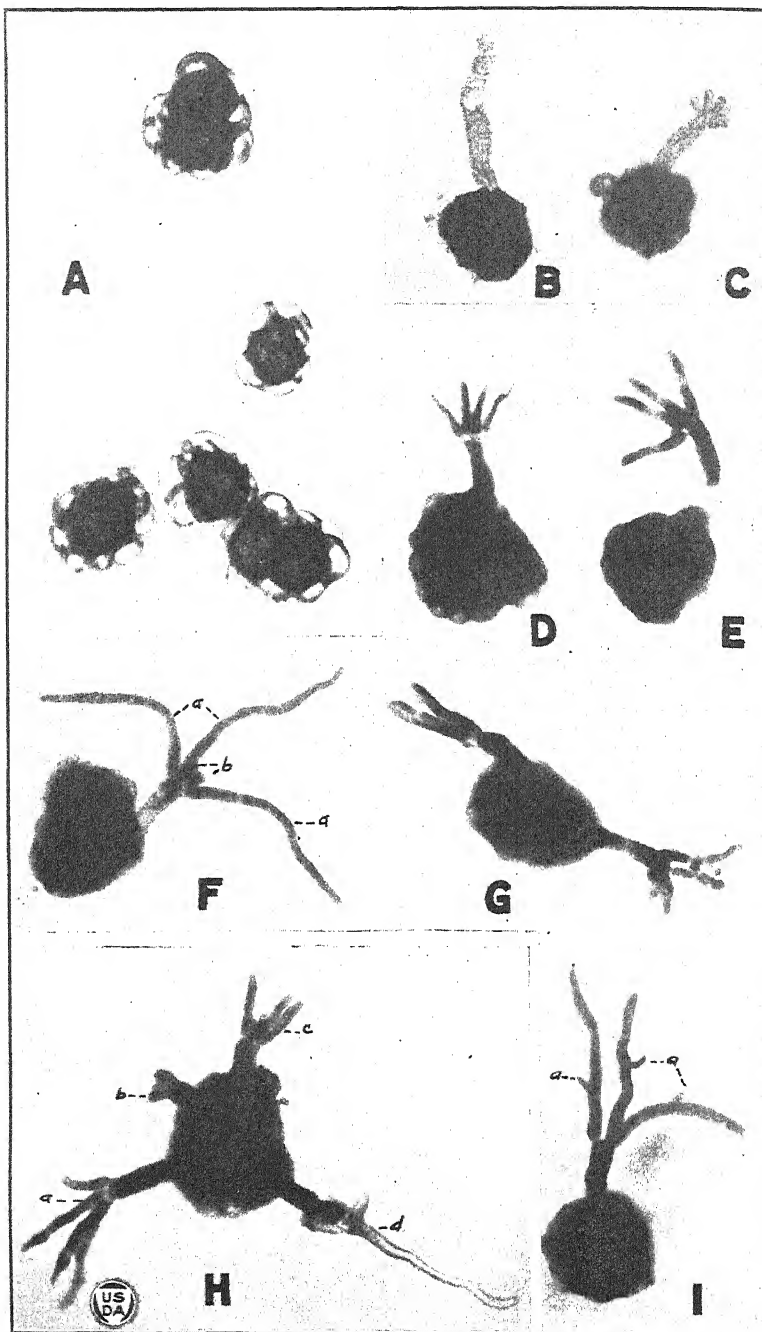
A.—Upper leaves of a wheat culm infected with *Urocystis tritici*, showing the long stripes, or sori.

B.—An enlarged portion of a wheat leaf infected with *Urocystis tritici*, showing the smut sori.

C.—Flag smut lesions on the lower glumes of an infected wheat head (*a*), and on the leaf sheath (*b*).

D.—Lesions of *Urocystis tritici* on the lower glumes (*a*), the rachis (*b*), and the upper part of the culm (*c*).







## PLATE 2

### Germination of spores of *Urocystis tritici*.

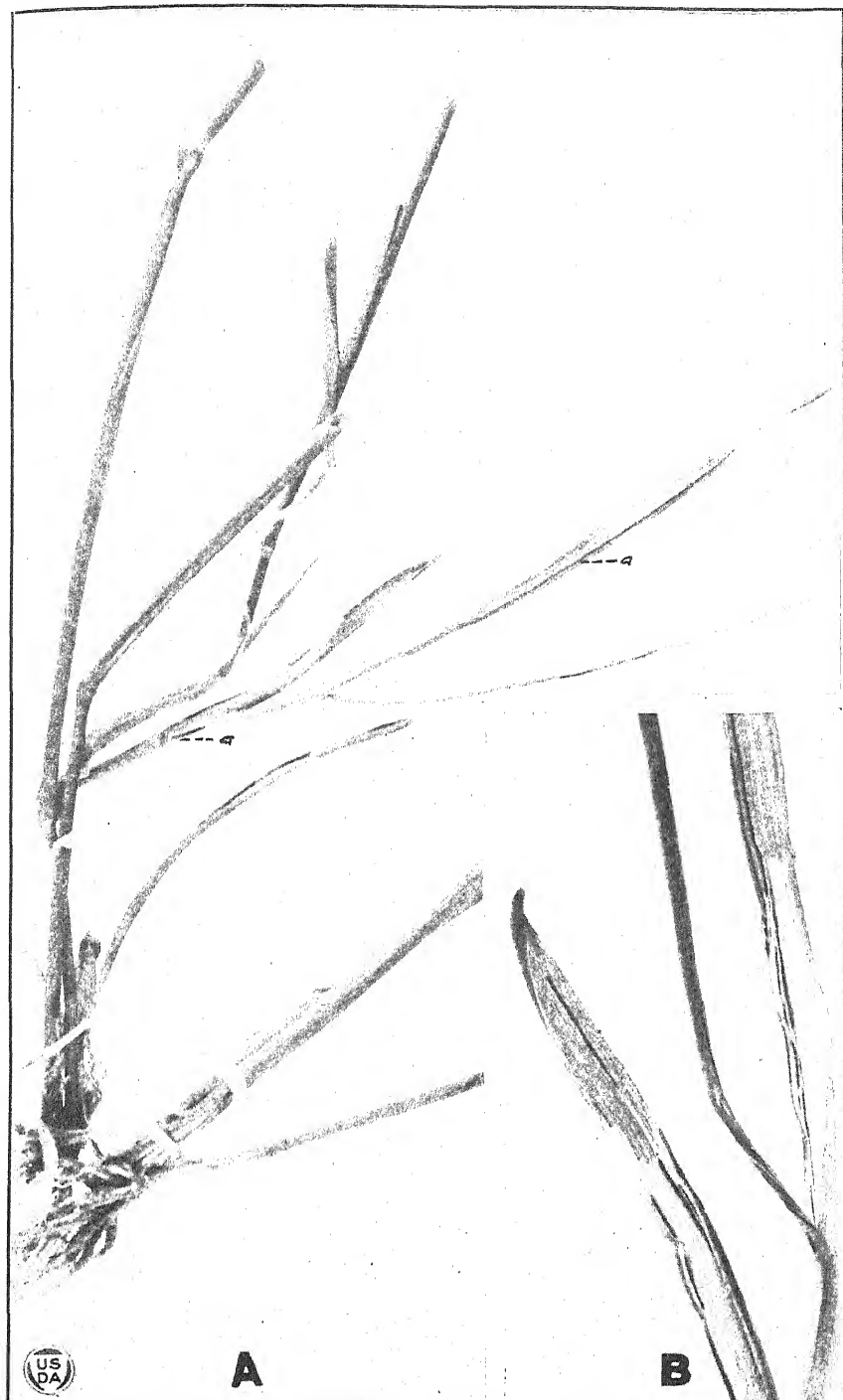
All figures were photographed with 4-mm. objective and No. 10 ocular by Miss Ruth Colvin, Office of Fruit-Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture.

- A.—Spore balls, each with one or two spores, invested by sterile cells.
- B.—Germinating spore with promycelium.
- C.—Germinating spore with sporidia just forming at apex of promycelium.
- D-E.—Germinating spores with sporidia.
- F.—Sporidia elongating, forming the so-called infection threads (a). Two sporidia (b) did not elongate.
- G.—Spore ball with two spores germinating, each with sporidia at the apex of the promycelium.
- H.—Spore ball with four spores in different stages of germination; (a) sporidia beginning to elongate, (b) sporidia just forming, (c) sporidia formed, and (d) sporidium elongated to form an infection thread.
- I.—Three sporidia, each with a secondary sporidium.

PLATE 3

A.—A cut-back plant of Comeback wheat showing an infected shoot (*a*) arising from the second node of one of the culms.

B.—An enlargement of a portion of the secondary shoot (*Aa*), showing the typical elongated smut sori.





# STUDIES ON THE PARASITISM OF *UROCYSTIS TRITICI* KOERN., THE ORGANISM CAUSING FLAG SMUT OF WHEAT<sup>1</sup>

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## INTRODUCTION

Flag smut of wheat is a disease which now occurs in a number of the important wheat-growing regions of the world. It has been known to exist in certain countries for many years, in others it has appeared for the first time only recently. In some countries and in some localities, it is not regarded as a very serious disease of wheat, but in others it is a disease of major importance, and its menacing spread is viewed with some alarm.

The disease has been studied by investigators from time to time, but many essential points are lacking in our knowledge of the reaction of the pathogene to its environment and its relationships within the host. The present studies, therefore, were directed toward an elucidation of some of these phases of the parasitism of the causal organism, *Urocystis tritici* Koern.

## HISTORY, GEOGRAPHIC DISTRIBUTION, AND ECONOMIC IMPORTANCE

Flag smut is one of the most destructive diseases of wheat. Under certain conditions it may exist in a locality for several seasons before its presence is suspected; and sometimes the disease has only been discovered after it has become epidemic.

McAlpine (29)<sup>3</sup> states that flag smut was first recorded from South Australia in 1868, although there is evidence that it was fairly widespread before that time. The disease has now been reported in Australia from Queensland, New South Wales, Victoria, and South Australia. There is as yet no record of its occurrence in Western Australia or Tasmania.

The disease was reported by Hori (18) in Japan in 1895, and Sydow and Butler (47, p. 427) recorded it from Lyallpur, India, in 1906. Reed and Dungan (42) report that it occurs in southern Europe. Dr. E. C. Stakman collected wheat affected with flag smut at Rieti, Italy, June 21, 1922.

<sup>1</sup> Received for publication Nov. 23, 1923. The investigation herein reported was carried on while the writer was a Fuller traveling research scholar of the University of Sydney, Australia. It was made possible by cooperative arrangement with the Bureau of Plant Industry, U. S. Department of Agriculture. The work was done at the University of Minnesota.

<sup>2</sup> The writer takes pleasure in acknowledging his indebtedness to Dr. E. C. Stakman, of the Department of Plant Pathology, University of Minnesota, for advice and helpful criticism during the progress of the investigation. The writer also is indebted to Marion A. Griffiths, of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, who is conducting studies on flag smut, for suggestions and access to her notes in June, 1922.

<sup>3</sup> Reference is made by number (*italic*) to "Literature cited," pp. 487-489.

Putterill (40) found the disease in the Marico district of the Transvaal, South Africa, in 1920. He states that it has probably been present a number of years and that, although the total loss is not great, 50 per cent of the plants may be affected in some fields. Flag smut also is known to occur in China (49, 50).

Flag smut was found in the United States for the first time in May, 1919, according to Humphrey and Johnson (19). It was found then in a number of fields in the vicinity of Granite City, Madison County, Ill. As a result of a survey made in 1920, the disease was found over an area of approximately 47 square miles (51). In 1921 the quarantine zone was extended to include 61 square miles. It is pointed out, however (45), that this is not necessarily due to spread of the disease but that a more intensive survey was possible in 1921.

The relative importance of the disease varies considerably in the various countries in which it is now known to exist. It would appear that in Australia the conditions necessary for the perpetuation of the pathogene have been especially favorable, for in that country flag smut is of the greatest importance.

In 1891, Cobb (9) stated that the disease was a serious plague. Brittlebank (4) in 1920 states that the prevalence of flag smut has increased to an alarming extent in recent years throughout the wheat-growing areas of Victoria, Australia. He states that since stem rust may ruin a crop in a few days under favorable weather conditions, many have considered it as the most destructive disease of cereals in Australia, but flag smut annually takes a toll of from 5 to 70 per cent of the crop, so that the total annual loss from rust sinks into insignificance when compared to that caused by flag smut.

Bartlett (2), in a report on a district wheat crop competition in New South Wales, Australia, in 1922, states that flag smut considerably reduced the yield of many crops, and mentions losses as high as 30 per cent of the crop in certain fields.

The amount of damage caused by the disease in any particular locality in a given year often may be closely correlated with environmental conditions and cultural practices. Hence, although it is not possible to give an accurate estimate of the total losses due to flag smut in Australia, there is sufficient evidence to show that the disease is widespread in the States concerned, and fairly conservative estimates place the average annual loss for Australia at 3 per cent.

The situation in India is one of great interest. Butler (7) states that the disease is confined to the Punjab. It has been many years since it was first recorded from that country, and, although apparently there have been opportunities for the disease to spread to other localities, it has not been found in any other wheat-growing areas.

Haskell, according to Stakman (45, p. 162-164), reports that the disease may cause considerable damage in western Illinois, where as many as 25 per cent of the plants have been diseased in certain fields. However, although the known region of infestation is apparently increasing, the disease evidently is being held in check or even reduced on individual farms.

## THE DISEASE

### SYMPTOMS

The first symptoms are more or less elongated grayish or dull white stripes on the young leaf. These stripes are slightly raised above the

general surface and are usually first apparent on the lower side of the leaf. They vary from a few millimeters to many centimeters in length (Pl. 1, B) and usually are between the vascular bundles, although occasionally they appear between the vascular bundle and the epidermis. More rarely the peduncle (neck) and inflorescence are attacked (Pl. 1, C). The stripes change from white to dull gray in color, usually from three to four days after the first appearance of the lesions.

The stripes represent individual sori of spores. These sori may remain unbroken for several weeks or less, depending on environmental conditions. The epidermis eventually ruptures and exposes the black mass of spores within. The lesions may appear on plants of practically all ages. McAlpine (29) reports the first production of new spores on plants 40 days old. The earliest production of spores observed by the writer occurred on the fifth leaf of a young seedling 29 days after inoculation. Occasionally the parasite gains the ascendancy just as the culms of the host begin to elongate rapidly.

Usually the organism causes considerable stunting of the plant. The leaves become characteristically incurled and twisted, and the plant may be considerably deformed (Pl. 1, D). Even if heads are formed on affected culms, they usually are empty or produce only badly shriveled kernels. Plants which are severely attacked when young may be killed before the unaffected plants are mature; hence their loss easily may be overlooked at harvest time. Frequently not all of the culms are equally severely affected. Many instances have been observed in which normal culms have grown to maturity, although others have been completely destroyed by the parasite. In studies on such plants, under greenhouse conditions, it was observed that these unaffected shoots were those first produced by the plant and that the later-tillering shoots frequently became infected.

#### THE CAUSAL ORGANISM

Flag smut of wheat is caused by *Urocystis tritici* Koern. It was first designated by Wolff (57) in 1873 as *U. occulta* (Wallr.) Rab., and the early records of the disease refer to it under this name. Koernicke (23, Bd. 16, p. 33-34), in 1877, first described it as a separate species. The morphological and physiological differences between *U. occulta*, which causes stem smut of rye, and *U. tritici* were again noted by McAlpine (29).

Koernicke (23), McAlpine (29), and others have described the morphological characters of the spores of *Urocystis tritici*. The latter may occur singly or in spore balls, which contain a variable number of spores, although from two to three spores are found most frequently in the ball. The spore balls are dark brown in color and variable in shape, though most frequently globose. They usually vary from 18 to 52 $\mu$  in length and from 18 to 45 $\mu$  in breadth. The individual spores are spherical or somewhat oval in shape and vary within the size limits of 12 to 16 $\mu$  by 9 to 12 $\mu$ . The spores which occur singly, and the spore balls also, are most frequently completely invested with a layer of sterile peripheral cells, pale brown in color, globose or ellipsoid in shape, and of dimensions 7 to 10 $\mu$  by 5 to 9 $\mu$  (Pl. 3, H).

McAlpine (29) has described some of the features of the germination of the spores. The writer (35) has recently described further phenomena

which may occur during germination. Each spore may produce a promycelium, usually about  $30\mu$  in length, which characteristically produces several prolongations which are usually considered sporidia. The sporidia are usually  $30$  by  $5\mu$  in size. A variable number may be produced on the promycelium, although from two to four occur most frequently. These structures may be formed under the surface of the medium in which the spores are germinating, at the surface of the medium, or in the air when only a portion of the spore is in contact with the substrate.

Wolff (57) mentions that the germ tubes produced by the sporidia of *Urocystis occulta* may become detached and then may be widely distributed by air. The writer has not observed that the sporidia or the sporidial germ tubes of *U. tritici* ever become detached. The sporidia characteristically germinate while attached to the promycelium. A germ tube is produced from the tip of the sporidium. It is fairly constantly  $3$  microns in width and under favorable conditions it may grow to a considerable length (from  $3$  to  $4$  mm. in solid nutrient media). The sporidia were observed to fuse occasionally, although this did not appear to be a characteristic feature at germination.

## PHYSIOLOGICAL STUDIES

### INTRODUCTION

There is little need to stress the importance of a knowledge of the conditions which govern germination of the spores of parasitic fungi. Other conditions being favorable, the severity of an outbreak of a disease frequently is determined in the first instance by the number of spores which cause infection of the host. As it has been shown that epidemics of flag smut frequently are traceable to the presence of viable spores in the soil, and as, under those conditions, control by present known methods of seed treatment is not possible, it appears especially desirable that we should have a more complete knowledge of the reaction of the pathogene to its environment, for the factors favoring germination and subsequent growth of the fungus must necessarily be closely related to those primarily responsible for the development of the disease.

The spores of *Urocystis tritici* have long been known to germinate capriciously. The writer (35) has referred to the difficulties experienced in obtaining results from spore germination studies and has described a method of stimulating spore germination. Spores which had been pre-soaked in water for several days germinated readily after the addition of small quantities of certain plant tissues. Tissues from wheat and rye plants were found to be the most effective.

Although not a characteristic feature of the spores of all smut fungi, those of many species do not germinate readily. Potter (38) speaks of the difficulty experienced in attempting to germinate spores of *Sorosporium reilianum* (Kuehn.) McAlp. In repeated tests in different seasons and at various times of the year, he noted only slight and irregular germination, and states that rarely did more than 15 per cent of the spores germinate.

Walker and Jones (52), in a study on *Urocystis cepulae* Frost, state that "the germination of the fungus spores has been so scanty that the effect of temperature upon the fungus has been necessarily limited to inoculation experiments with infected soil."



Stakman (43) records that neither Kühn nor Brefeld was able to secure germination of fresh spores of *Ustilago zeae* (Beck.) Ung., but that Brefeld secured germination in nutrient media, although the spores did not germinate in water until the following spring. Stakman found also that, with the exception of one spore lot, a rest period was required before the spores would germinate. In a review of germination studies on *Tilletia levis* Kühn and *T. tritici* (Bjerk.) Wint., the same investigator reports the uncertain and capricious germination observed by Prévost, DeCandolle, Tulasne, Kühn, Fischer von Waldheim, and Brefeld. Stakman (43) reports also that he was rarely able to secure germination of fresh spores, although he was successful in one instance (20 per cent being recorded) and then only when distilled water was used, but that after the spores had passed through a rest period of about eight months' duration almost 100 per cent of them germinated.

It is recognized that distilled water is not always a satisfactory medium for the germination of spores, but this apparently is not always a limiting factor, for it is sometimes as effective as a "nutrient solution," and sometimes even more effective.

#### MATERIAL AND METHODS

The methods used were essentially the same as those previously reported (35). Germination studies were made in Syracuse dishes containing up to 5 cc. of medium.

The spores used in practically all the tests were obtained from material collected in Illinois or from material produced in the greenhouses of the United States Department of Agriculture at Washington, D. C. This material was not more than 12 months old when used in the tests. Spores from material collected in Australia and Italy were used in a number of confirmatory tests made from time to time.

#### MATURATION

Reference already has been made to the fact that the age of the spore is sometimes a factor in determining its viability. A rest or maturation period apparently is necessary before the spores of many fungi can germinate. The teliospores of *Puccinia graminis* and the oospores of many Phycomycetes are familiar examples.

Davis (11), in germination studies on spores of *Ustilago striaeformis* (West.) Niess., states that the spores germinate when properly after-ripened. "The spores pass through an after-ripening period varying from 180 to 265 days." The period of germinability is from 53 to 210 days, and "nourishing solutions and decoctions are of no visible value in forcing the germination of these smut spores."

McAlpine (29) reports the result of germination studies with *Urocystis tritici* and has suggested that the age of the spore is a possible factor governing germination. He states: "Spores were taken from the wheat plant immediately after maturity and placed directly in water on a slide, but they did not germinate. With material, however, about a month old, and kept seven days on soil, a small proportion of the spores germinated in water after 24 hours." He obtained up to 40 per cent of germinating spores in material several months old.

The nature of this "after-ripening" period is not known, although it generally is assumed to be analogous to the after-ripening period of the seeds of many higher plants. It is possible that with many fungi this period is necessary in order to enable certain changes to take place in the protoplasmic contents of the spore or spore wall.

In experiments designed to hasten the after-ripening process in teliospores of *Puccinia graminis*, Thiel and Weiss (48) secured germination of the spores after preliminary treatment with dilute citric acid, several months before germination could be obtained by ordinary methods. They report the work of Crocker, Eckerson, Denny, and others on after-ripening in seeds and permeability of seed coats, in which it has been shown that germination may be delayed until certain definite physiologic changes have taken place in the embryo, or, in other cases, until the seed coats become permeable to water and oxygen.

Thiel and Weiss state that the stimulus to germination of teliospores apparently was not the result of increased permeability of the spore wall, but rather that the citric acid appeared to function as a specific activator of the protoplasm. However, similar treatment of spores of *Urocystis tritici* did not materially increase the amount of germination.

Harrington (14) has discussed the effect of artificially drying by heat in hastening the germination of seeds of various cereals which had not after-ripened. Many investigators have mentioned the beneficial effects of such a procedure. A somewhat similar effect after drying in vacuo or over sulphuric acid also has been observed.

While studying the effect of temperature and relative humidity on the viability of the spores of *Urocystis tritici*, the writer obtained some interesting results on the effect of drying fresh spores which had been produced under greenhouse conditions.

Certain infected leaves were closely watched for the change of color in lesions. Two days after the lesions had changed from a whitish gray to a dull, leaden-black color, the infected leaf was removed, cut into strips about 1 cm. in length, and then dried for 48 hours at room temperature (18° to 22° C.) in a dessicator over concentrated sulphuric acid. At the end of this period the spores were shaken out from the leaf section onto the surface of a few cubic centimeters of distilled water, in the manner already referred to, and after three days small quantities of young wheat tissue were added. Eighteen hours later, from 60 to 70 per cent of the spores had germinated. In control dishes, spores obtained from the fresh, undried lesions failed to germinate on the addition of similar amounts of young tissue. Four weeks later, when the infected plants were removed, the sori in the remaining leaves were still unruptured.

It is not known whether there was any effect on the internal composition of the spores or whether the treatment merely acted on the spore envelope, possibly removing certain substances from its surface and thereby facilitating the ingress of water necessary for germination. However, it apparently had the effect of shortening the maturation period, which previously had been considered as essential before the spores would germinate.

It is thus evident that caution should be exercised in stating that certain definite maturation periods are necessary unless the environmental conditions are known. Attention is drawn to this fact elsewhere in this paper in a discussion of the effect of chemical stimulants on the spore.

## ACTION OF PLANT ROOTS

It is noted by the writer (35) that the addition of uninjured wheat seedlings to dishes of distilled water containing presoaked spores stimulated germination almost to the same extent as did the addition of small portions of plant tissue.

Several investigators have reported that the presence of the host may profoundly affect germination of spores of parasitic organisms.

Brown (5) has observed that drops of distilled water placed on the surface of leaves and floral structures of a number of plants stimulated the germination of spores of *Botrytis cinerea* Pers. This stimulation was correlated with an exosmosis of electrolytes from the host tissue, as indicated by increased electrical conductivity of the drop.

Chupp (8) states "that the spores of *Plasmidiophora brassicae* Wor. do not germinate at ordinary room temperatures ( $16^{\circ}$  to  $21^{\circ}$  C.) but do so readily when placed in test tubes on agar with young cabbage seedlings. The presence of the host seems in some manner to exert an influence which to a certain extent takes the place of that offered by a greater amount of heat."

Among some phanerogamic parasites, also, there is evidence that the presence of the host may be necessary in order to supply the stimulus for germination of the seed of the parasite. Duggar (12) has called attention to the work of Koch and Heinricher on germination of seeds of Orobanchaceae. Heinricher makes the interesting observation that the seeds of the parasite germinate in "periods of greatest humidity" when in the presence of the host plant. A substance excreted from the host plant evidently furnished the necessary stimulus, although its nature had not been determined.

More recently McWhorter (32) records the work of Kusano on *Aeginetia indica*, an Orobanchace parasitic on sugar cane. Kusano observed that "the seeds of the parasite lie in the soil for months waiting for the rootlets of some holophytic plant to come near enough to furnish the stimulus necessary for their germination. They can not germinate until the actual contact with a suitable host is attained. The roots of the wrong host will make the seeds start germinating, but they can not complete germination until the roots of a suitable host find them."

In further tests with spores of *Urocystis tritici* it has been found that uninjured seedlings of other plants, namely, field peas and beans, stimulated the germination of spores which had been presoaked in distilled water, although the percentage of germination was not so great as in the instances where wheat or rye seedlings were used (Table I). These spores did not germinate as rapidly as those of the cultures in which cut tissues were used. It is an interesting fact, however, that so many spores germinated, and particularly that nonsusceptible plants should have such a marked influence in this respect. The results are given in Table I. The spores were sown on April 4, incubated at  $22^{\circ}$  C., and the different seedlings added on April 10.

TABLE I.—Effect of uninjured plant tissue on the germination of spores of *Urocystis tritici* presoaked in distilled water

Number of dish.	Plant.	Percentage of germination on—			
		Apr. 10.	Apr. 11.	Apr. 12.	Apr. 13.
96	Wheat .....	0	98	.....	.....
97	.....do.....	0	90	.....	.....
98	.....do.....	0	85	.....	.....
99	Rye .....	0	87	.....	.....
100	.....do.....	0	91	.....	.....
1	.....do.....	0	72	.....	.....
2	Field pea .....	0	Trace.	Trace.	.....
3	.....do.....	0	21	.....	.....
4	.....do.....	0	2	.....	.....
5	Bean.....	0	5	.....	.....
6	.....do.....	0	45	.....	.....
7	.....do.....	0	11	.....	.....
8	Control of distilled water.....	0	0	0	0
9	.....do.....	0	0	0	0

It is possible that such a relationship may have a very fundamental bearing on the question of the epidemiology of the flag-smut disease. The soil solution is of complex and varying composition. Laboratory studies on the germination of spores in soil extract solutions also have shown that the spores will germinate more profusely on the addition of young tissues of various plants. Hence, although it is not possible to duplicate soil conditions in the laboratory, it would appear that at certain times certain facts observed under controlled conditions in the laboratory may at least be duplicated in the field. When there is adequate moisture in the field, the presence of actively growing plant roots alone may be expected to stimulate the germination of many spores of *Urocystis tritici* and thus reduce the amount of viable inoculum between successive crops of the susceptible host. It is a matter of common observation that, under normal conditions, when supplied with an adequate supply of oxygen, plant roots excrete carbon dioxide; when insufficiently supplied with oxygen, the products of anaerobic respiration are carbon dioxide, alcohol, and various organic acids, such as formic, acetic, and lactic.

It is apparent that the most marked stimulation of the spores is to be expected when the environment causes a certain amount of anaerobic respiration of the host roots. It is not known whether the organic acids or the alcohols are primarily responsible for the germination observed, or whether certain other constituents of the plant are effective, for subsequent studies have indicated that small amounts of other volatile substances are effective in this respect also.

#### EFFECT OF PLANT TISSUE EXTRACTS

Reference has been made to the fact that young tissues of a number of plants stimulated germination of presoaked spores of *Urocystis tritici*.

A number of investigators have observed that plant decoctions form a very suitable medium for the germination of spores of many organisms. Anderson (1) has shown that the germination of pycnosporos of *Endothia parasitica* Murr. is slight and uncertain when sown on tap water, rain

water, or distilled water, but that they germinate readily in a decoction of chestnut bark. The action of chestnut bark was not specific, for decoctions of material from trees belonging to other genera, and various nutrient agars, produced the same effect.

Leach (26) found that spores of *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cavara germinated poorly in distilled water, but readily when small portions of the host tissue were present.

Whitehead (55) reports that spores of *Urocystis cepulae* Frost germinate slowly when sown in water, but much more rapidly and vigorously when they are sown in onion juice.

In studies on the longevity of spores, Duggar (12) has shown that the spores of *Aspergillus flavus* Lk. and *Sterigmatocystis nigra* Britz would not germinate on the surface of distilled water, but when they were transferred to a decoction of beans they germinated readily. Spores of this kind, however, are known to germinate readily on a wide range of nutrient media.

Although plant tissues were so effective in stimulating the germination of spores of *Urocystis tritici*, the optimum effect could not be produced when the tissue was sown with the spores but only after the latter had gone through a "presoak" period. It is suggested that such procedure is necessary because of the nature of the spore wall and also because of the nature of the activating agent. The exact nature of the stimulatory substances in these instances is not known, but from subsequent tests it appears possible that a number of more or less simple substances may be operative. In the earlier tests several sections of wheat tissue were added to a few cubic centimeters of distilled water. Plant tissue decoctions also were effective but these had to be in a suitable dilution.

The concentration of the wheat tissue extracts will vary somewhat according to the conditions under which the seedlings are grown. In the following studies, seed of a number of common wheats was sown between sheets of moist blotting paper at 20° C. Six days later entire seedlings were ground in a meat chopper and the sap expressed with the aid of a hand press. It was found that a concentration of approximately 1 part per 10,000 was the most favorable for germination of the presoaked spores. At higher concentrations germination frequently was delayed. Promyelia sometimes were stunted and distorted and occasionally no spores germinated.

The writer has recorded (35) that a steam distillate of a plant infusion also increased spore germination when satisfactory concentrations were used.

Brown (6) has recorded the stimulative action of certain volatile products arising from the tissues of a number of plants on the germination of spores of *Botrytis cinerea* Pers., *B. parasitica* (allii), *Monilia fructigena* Pers., *Penicillium glaucum* Lk., *Fusarium* sp. and *Colletotrichum*. He concludes that "while no exact rule can be laid down in this respect, one can say broadly that some plants give good stimulation, others less so. In some instances volatile materials appeared to inhibit the germination of the spores." He also studied the effects of various chemical substances on the germination of spores of *Botrytis* and states that "a rough comparison was made of several ethyl esters by testing the effect of a few cubic centimeters of a saturated watery solution of each and of a tenth dilution of the same on the germination of *Botrytis* in Petri dishes. In all cases inhibition was shown in the presence of the saturated solutions, while stimulation appeared over the tenth saturated solutions of ethyl acetate, malate, and citrate. Any further comparison was not attempted. . . ."

Since it had been shown that the materials which stimulated germination of the spores of *Urocystis tritici* were volatile in steam, tests were made to determine if these materials would volatilize at lower temperatures.

Presoaked spores of *Urocystis tritici* were placed on the surface of a few cubic centimeters of distilled water in Syracuse dishes. The dishes were then placed in a vessel which contained a small quantity of expressed sap of wheat seedlings, and the container was then sealed. After 18 hours at 22° C., over 50 per cent of the spores had germinated in each of the three trials. There was no germination in the controls. It is possible in these cases that the stimulus may have been derived from some of the original constituents of the plant sap, or it may have been derived from some of the products of its decomposition, possibly organic acids, esters, or alcohols.

The spores of *Urocystis tritici* did not germinate readily in solid media, although many different nutrient substrata were used. However, presoaked spores germinated in wheat plant extract agar or in agar containing appropriate concentrations of the distillate from such extracts. Attempts were made to sterilize the surface of the spores by washing them in one-half per cent solution of copper sulphate for periods up to two minutes before transferring them to sterile distilled water for presoaking and subsequent transference to the solid media.

Under these conditions it was not found possible to eliminate entirely all bacterial contamination. However, the spores produced normal sporidia, which in turn produced germ tubes which grew to a length of 4 mm. after four weeks at 7° C. The manner of growth was similar to that previously recorded in liquid media. The protoplasmic contents remained segregated at the tip, while the remainder of the germ tube became septate and apparently was devoid of contents. There appeared to be no increase in the amount of protoplasm in the germ tube.

It is not known whether such germ tubes are capable of producing infection. There is the interesting possibility, however, that the infection capabilities of the organism may be increased by such an extended growth period under soil conditions.

#### NATURE OF STIMULUS

The identification of the activating constituents of the distillates of wheat-plant decoction is very difficult. The stimulus appeared to be most efficient when the distillates were highly diluted. The distillates obviously are complex in composition and the individual components might be expected to function very differently on spores exposed to their action. Several attempts were made to determine if the activating substances belonged to any general group of chemical substances. "Functional" distillates were used, i. e., distillates in which presoaked spores germinated readily. These were acidified with sulphuric acid and redistilled. The original distillates also were made alkaline with barium hydroxid and then redistilled. Subsequent germination tests with these products of the second distillation showed that in each case they were as potent as the original distillate.

It would thus appear either that a number of the original constituents of the wheat plant decoction were operative or that the stimulus came from some ingredient which was unaffected in either acid or alkaline solution.

It was thought possible that small quantities of volatile oils were the effective constituents of the wheat-plant distillates. A series of rather empirical tests then were designed to test the action of a number of volatile oils on the germination of the spores of *Urocystis tritici*.

The spores were sown on the surface of from 5 to 7 cc. of distilled water at from 18° to 22° C., as in the ordinary germination tests. A minute quantity of each substance was then applied on the point of a needle to each dish of spores. A film formed on the surface of each dish, although slightly different amounts of oil were added in each instance.

The materials which stimulated germination of the spores are shown in Table II. Similar tests also were made with salicylaldehyde, butyric acid, acetone, benzol, oil of thyme, ethyl alcohol, methyl acetate, phenol, benzoic acid, lactic acid, acetic acid, and citric acid. After 24 hours at 22° C., from 80 to 90 per cent of the spores treated with the first three substances had germinated. Less than 1 per cent of the spores treated with the other substances germinated.

Several other essential oils, namely, oil of hemlock (bornyl acetate 30 per cent), oil of lemon (*d*-limonene and citral), eucalyptus (cineol 50 per cent), and eugenol failed to stimulate germination.

No spores germinated in the control dishes. It thus appeared that a large number of substances, in appropriate concentrations, might furnish the necessary stimulus for germination of spores of *Urocystis tritici*.

It is obvious that under the conditions of the experiment (in which the Syracuse dishes are packed in the form of a nest, each dish forming a cover for the one immediately below it), the containers are not air-tight and there might be considerable volatilization of certain of the oils during the period in which the spores might be expected to germinate.

TABLE II.—The effect of surface films of volatile oils on the germination of spores of *Urocystis tritici* presoaked on surface of distilled water seven days at from 18° to 22° C. and oil applied on December 6

Number of dish.	Oil.	Percentage of germination on—		
		Dec. 7.	Dec. 8.	Dec. 9.
26	Balsam peru (cinnamein, 50-65 per cent).....	67	.....	.....
28	Oil of tansy (thuyol alcohol, 17 per cent).....	29	.....	.....
53	Oil of cassia (cinnamic aldehyde, 75 per cent; trace lead and copper).....	15	.....	.....
34	Oil of verbena (citral, 50 per cent).....	Trace.	.....	.....
35	Oil of bitter almonds (benzaldehyde, 85 per cent).....	91	.....	.....
36	Oil of camphor (safrol, eugenol).....	Trace.	.....	.....
37	Oil of bergamot.....	Trace.	.....	.....
38	Oil of lavender.....	Trace.	.....	.....
39	Oil of pine needles (30 per cent bornyl acetate).....	Trace.	.....	.....
40	Control of distilled water.....	0	0	0
41	.....do.....	0	0	0

A study was then made of solutions of certain of the above activating substances to determine the optimum concentrations. Certain of these organic substances are quite soluble in water but are highly volatile (e. g., acetone B. P. 56.48° C., alcohol 64°, ethyl acetate 77°); others again are only slightly soluble, while still others are definitely soluble, even though only in slight amounts and less volatile.

In a number of tests benzaldehyde greatly stimulated germination. Its solubility is slight (0.3 part per 100 parts of water at 20° C.), but its boiling point is comparatively high (179.9°). Thus, it appeared to be suitable for making comparative germination tests under laboratory conditions. A half-saturated solution of benzaldehyde in distilled water was prepared and this was used as a basis for subsequent dilutions. The results of one test are shown in Table III.

At a concentration of 3 parts per 200,000, germination was delayed and subsequent growth of promycelia and germ tubes showed characteristic dwarfing and other abnormalities. This experiment was again repeated in duplicate and it was found that the most favorable concentration was again 3 parts per 2,000,000. It was found that spores subjected to concentrations of 3:20,000 for 18 hours at 20° C. revived when transferred to distilled water and germinated in a normal manner, but those exposed to concentrations of 3:2,000 for the same period apparently had been killed.

Germination at the optimum concentration was quite similar to that induced by the addition of small quantities of plant tissue as in previous experiments. In the latter instances, however, the germ tubes eventually grew further than did those in the benzaldehyde solutions.

TABLE III.—The effect of a solution of different concentrations of benzaldehyde in distilled water on the germination of spores of *Urocystis tritici* presoaked at from 18° to 22° C. for six days prior to test, and transferred to solutions on January 8

Number of dish.	Concentration.	Percentage of germination on—		
		January 8.	January 9.	January 10.
85	3:20,000.....	0	0	0
86	3:20,000.....	0	0	0
87	3:200,000.....	0	Trace.	.....
88	3:200,000.....	0	do	.....
89	3:2,000,000.....	0	86	.....
90	3:2,000,000.....	0	80	.....
91	3:20,000,000.....	0	Trace.	.....
92	3:20,000,000.....	0	1	.....
93	3:200,000,000.....	0	0	.....
94	3:200,000,000.....	0	0	0
95	Control in distilled water.....	0	0	0
96	.....do.....	0	0	0

A similar test with butyric acid resulted in germination of presoaked spores when sown in concentrations ranging from 1 part in 100,000 distilled water (by volume) to 1 part per million. The optimum concentration for germination of presoaked spores appeared to be 1 part per 500,000.

The results of similar tests with other materials more or less soluble in water were not as striking as those given above. It is possible that they may have been less effective in any case, or that the effective range of concentration was more restricted. In some cases, however, it would appear that because of their great volatility the concentrations are more readily changed during the process of preparation of the dilutions and during the course of the experiment; hence their effect would not be so evident in experiments such as this.



## RELATION TO PRESOAKING

Reference already has been made to a method of procedure which involved presoaking of the spores and subsequent addition of a stimulatory agent, which resulted in an optimum germination of the spores of *Urocystis tritici*.

Harrington (14) recently has reviewed the results of previous investigations on the presoaking of seeds of certain cereals and its relation to germination and subsequent growth of the plant. He also has studied the effect of presoaking "not after-ripened" seeds of cereals. Within certain limits the procedure had a beneficial effect. Many factor relationships apparently are concerned in the process, but the results indicated that changes in seed-coat permeability were chiefly involved. This author also mentions the work of Eberhardt, who has drawn the interesting conclusion that, although seed-coat permeabilities are increased, protoplasmic alterations also must be involved, as the effect was observed throughout the subsequent growing period.

In a test to determine whether constant association with benzaldehyde in solution would result in germination of spores, results were obtained which were analogous to those previously reported by the writer (35) when plant tissues were used.

In a few cases slight germination was noted after six days at 15° C., but in the majority of instances no spores germinated. These spores were viable, for they germinated on the readdition of the activating agent. It would appear that the activating agent had volatilized before it could penetrate and act upon the spore contents. This would explain why spores germinate more readily after presoaking than when sown directly on an activating solution. In the latter instance care has to be taken to prevent loss by volatilization, or allowances have to be made for it by using a more concentrated solution. In such a case there is danger that the more concentrated solutions may be more toxic to the spores.

Thus, it seems most probable that the period of presoaking of spores in water results in an increased permeability of the spore envelope, as the spores respond with apparently equal vigor to the action of a stimulus within a presoak period of from 3 to 10 days at 20° C. This applies equally to spores sown on the surface of water and those totally immersed. There is undoubtedly some action on the spore contents also, but this appears to be only slight until after a sufficiently long period, when the spores germinate without the action of any special stimulus.

## ACTION OF STIMULUS

The above results have indicated that many different substances stimulate the germination of the spores of *Urocystis tritici*. They differ widely in chemical composition, for they include acids, alcohols, ketones, aldehydes, and esters. There is no one individual substance which appeared to function as a specific activator of the protoplasm, such as was suggested by Thiel and Weiss (48) in their studies on teliospore germination.

It is possible that these substances function partly by increasing the permeability of the spore envelope, although this appears improbable, as the presoak period is not materially shortened by their use. It would appear more probable that they function mainly in increasing the permeability of the protoplasmic spore contents, and thus allow a more

ready ingress of the water necessary for germination of the spore. Without the action of the stimulatory materials, germination in distilled water either is delayed or may not occur at all. The exact nature of the action of these substances on the spores is not known. However, they all have the common property of reducing the surface tension of water.

Larson (24) has shown that the surface tension of the medium may have a very definite effect on the cultural habit of certain bacteria. Pellicle-forming bacteria, such as those of the *Bacillus tuberculosis* and *B. subtilis* groups, which habitually grow upon the surface of liquid media, will grow throughout the body of the medium when the surface tension is reduced from 59 dynes (the S. T. of ordinary broth) to from 40 to 45 dynes. The suggestion is made that the bacteria grow throughout the medium when they are properly "wetted."

In a later paper Larson and Montank (25) observe that the pathogenicity and viability of the tubercle bacillus also are affected by the reduction of the surface tension of the medium. They suggest that "when the tubercle bacilli are growing diffusely throughout the medium they are wetted and, therefore, when introduced into the animal body, are penetrated by the antibodies or bactericidal substances present and destroyed."

The viability of these organisms is thus dependent on their degree of permeability to bactericidal substances, and this permeability is correlated with a reduced surface tension of the medium.

It has been considered that changes in surface tension of the medium sometimes affect the germination of fungous spores. Many of the stimuli recorded by Duggar (12) in his spore germination studies—e. g., alcohol and organic acids—are known to be depressants of the surface tension of water. He states, however, that "attempts to increase the surface tension by means of small quantities of oil in the water gave only negative results."

Melhus and Durrell (34, p. 132-134) recorded stimulation of germination of urediniospores of *Puccinia coronata* Cda. by the use of vaseline and paraffin oil. They state that "just how this oil brings about this stimulation is not known, but it is apparent that the surface tension of the water is changed."

Studies by the writer indicated that changes in the surface tension of the medium appeared to have but little effect on the germination of spores of *Urocystis tritici*. Aqueous solutions of sodium ricinoleate and sodium oleate were used. The surface tensions ranged from 72 dynes to 38 dynes, but no significant results were obtained. It was found, however, that very dilute solutions of a number of soaps resulted in germination of the spores (Table IV). Observations were not made on the surface tension of these solutions which were more effective than distilled water but less effective than many of the stimulatory substances discussed above.

Sodium stearate solution, 1 part to 4,000,000, had the most marked effect. It is evident that in such a concentration there is only a very slight change in the surface tension of the fluid as measured against air.

It is not known whether the soap or the hydrolytic products were effective in these cases. The spores failed to germinate in very dilute solutions of potassium hydrate, and hence the soap itself or the fatty-acid radicle appears to have been responsible for the change.

Observations were made on the surface tension of the solutions of benzaldehyde and of butyric acid when in concentrations suitable for the germination of the presoaked spores of *Urocystis tritici*.

TABLE IV.—The effect of soap solutions on the germination of spores of *Urocystis tritici*, 11 months old, sown February 15 at from 16° to 22° C.

Number of dish.	Medium.	Percentage of germination on—						
		Feb. 21.	Feb. 22.	Feb. 23.	Feb. 24.	Feb. 25.	Feb. 26.	Feb. 27.
55	Dist. H <sub>2</sub> O.....	o	o	o	o	o	o	o
56	.....do.....	o	o	o	o	o	8	.....
58	Ivory soap, 1:40,000....	o	o	o	10	.....	.....	.....
64	Ivory soap, 1:400,000...	o	o	o	Trace.	.....	.....	.....
70	Ivory soap, 1:4,000,000...	o	o	o	Trace.	.....	.....	.....
59	Pot. palmitate, 1:40,000.	o	o	o	7	.....	.....	.....
65	Pot. palmitate, 1:400,000.	o	o	o	30	.....	.....	.....
71	Pot. palmitate, 1:4,000,000.	o	o	o	53	.....	.....	.....
60	Sod. stearate, 1:40,000...	o	o	o	o	o	o	Trace.
66	Sod. stearate, 1:400,000...	o	o	o	92	.....	.....	.....
72	Sod. stearate, 1:4,000,000.	o	86	.....	.....	.....	.....	.....
61	Pot. stearate, 1:40,000...	o	o	o	10	.....	.....	.....
67	Pot. stearate, 1:400,000...	o	o	o	15	.....	.....	.....
73	Pot. stearate, 1:4,000,000.	o	o	o	o	o	o	o
62	Sod. oleate, 1:40,000...	o	o	o	Trace.	.....	.....	.....
68	Sod. oleate, 1:400,000...	o	o	o	20	.....	.....	.....
74	Sod. oleate, 1:4,000,000...	o	o	o	Trace.	.....	.....	.....
63	Sod. palmitate, 1:40,000.	o	o	o	o	o	o	Trace.
69	Sod. palmitate, 1:400,000.	o	o	o	o	o	o	o
75	Sod. palmitate, 1:4,000,000.	o	o	o	o	o	o	o

The observations were made with a Du Noüy surface tension apparatus in which the force necessary to pull a ring from the surface of the liquid was measured as a function of the torsion of a wire. No significant differences were observed between the surface tension of water and that of benzaldehyde in a concentration of 3 parts per 2,000,000 parts of water. The surface tension of butyric acid in water solution (1 part per 100,000) differed from that of water by only 3 to 4 dynes. It would appear that the surface tension of these fluids when measured against air is not a satisfactory criterion for comparison of their effect on the germination of the spores of *Urocystis tritici*.

Stiles (46) has extensively reviewed the evidence on the nature of permeability of plant tissues and has reported further experiments on the action of alcohols and certain electrolytes on the permeability of such tissues. Both the cell wall and protoplasmic cell contents are concerned in the process, but the constitution of the protoplasm is of fundamental importance. Protoplasm is considered primarily as a complex colloidal system in which the constituents are continually changing their relationships in reference to one another as a result of the action of various physical and chemical forces to which they are exposed.

In such a system surface action is of paramount importance, and surface phenomena are considered to play an important part in changing the permeability of the cell. In discussing the subject of surface tension, this author calls attention to the fact, already noted by Koltzoff and Vernon, that, although the surface tension of a solution be measured against air, in the experiment the surface tension is acting against the outer layer of protoplasm, and it is not possible to determine the surface tension between two immiscible liquids by knowing only the surface tension of each against air.

Hence, it is possible that entirely new relationships may occur within the protoplasmic cell contents as a result of the action of some substance—e. g., benzaldehyde, certain organic acids, ketones, or alcohols—even though the possibility of this action may not always be indicated by a specific change in the surface tension of the solution.

Price (39) has made observations on the protoplasmic condition of certain fungous spores. He used the method of dark ground illumination in studies on dormant and germinating spores of *Mucor* sp., *Melamp-sora rostrupii* Wagner, *Triphragmium ulmariae* (Schm.) Link. and *Phragmidium disciflorum* James, and has adduced further evidence on the colloidal nature of the plasmic contents of these bodies. He is of the opinion that the entire spore content is protoplasmic and of the nature of an emulsoid colloid. In the dormant spore the protoplasm is gellike, and at germination the most important change is the transformation from the gel state to a hydrosol.

It would appear, from the fact that so many substances stimulate the germination of spores of *Urocystis tritici*, that their action is such as to change the physical condition of the spore contents, facilitating a change of phase similar to that observed by Price and thus finally increasing the permeability of the spore contents.

Only small quantities of the stimulating materials were necessary, but it is possible, even in the high dilutions observed, that, owing to their physical properties, they readily concentrated at the surfaces and that there may have been a quantitative relationship between the actual amount of the substance in solution and the number of spores which responded to the action of the stimulus. This, however, was not apparent in the dilution tests.

It has been pointed out that the spores of *Urocystis tritici* ordinarily germinate slowly. Without the intervention of a stimulus, the spores may require up to 16 days at 20° C. before they germinate. The first stage of germination is generally considered to be the imbibition of water. The relatively impermeable spore wall permits the ingress of water only very slowly, but when the permeability of both spore wall and spore contents is increased, the spores germinate within a few hours. If the evidence justifies us in considering that the stimulatory substances probably cause a physical change of state in the protoplasmic cell contents and thus increase permeability, we might expect that this action probably is not specific for *U. tritici*. The spores of other fungi which do not germinate readily in water, or which seem to require a rest period before germinating, might also be stimulated by the same substances.

Preliminary tests were made with the spores of *Urocystis occulta*, *Colletotrichum lindemuthianum*, and the teliospores of *Puccinia graminis*. Evidence was obtained that they also respond to such treatment. Such results indicate that caution is necessary in concluding that definite rest or maturation periods are necessary for the spores of such organisms, without taking into consideration the nature of the environmental conditions to which the spores have been subjected.

The methods used in the studies of spore germination in *Urocystis tritici*, which involve the use of surface films or highly diluted solutions of stimulatory substances, would appear to indicate a fruitful mode of attack in studies on the germination of other forms. The practical importance of the method in its relation to pathogenicity studies with certain other fungous spores requires no further comment.

## RELATION TO HYDROGEN-ION CONCENTRATION

Many investigators have studied the effect of the acidity of the medium on the germination of fungous spores, but little is known concerning the behavior of the smut fungi in this connection.

Webb (54, p. 326) has stated that the spores of *Ustilago avenae* (Pers.) Jens. did not germinate readily in potassium phosphate solutions of different hydrogen-ion concentrations. In mannite solutions, however, the spores germinated within the range  $P_H$  2.4 to 8.2 with an optimum at  $P_H$  6.2. Sporidia were formed most abundantly from  $P_H$  5.4 to 7.0.

The writer has observed that the spores of *Urocystis tritici* did not germinate readily in potassium phosphate solutions, but they did so in certain of them after the addition of small quantities of a stimulatory agent.

Spores were sown in approximately 1½ per cent potassium solutions. Varying amounts of  $\frac{M}{5}$  potassium hydrate and  $\frac{M}{5}$  phosphoric acid were used in making a series in which the hydrogen-ion concentration varied from  $P_H$  2.4 to 9.6. Final selection of the solutions was made after potentiometer determinations of the hydrogen-ion concentrations.

Two series of observations were made. In the first the spores were sown directly in the solutions and five days later a film of benzaldehyde was added to each dish in the manner already described. In the second series the spores were presoaked in distilled water for five days before they were transferred to the phosphate solutions. Benzaldehyde was then added as in the first instance. The spores were nine months old at the time of testing, and the cultures were kept at from 20° to 22° C. throughout.

It is observed from Table V that the spores germinated within a rather restricted range of hydrogen-ion concentrations. When they were sown directly into the phosphate solutions, they germinated within the range  $P_H$  4.1 to 7.1. Presoaking before transfer appeared to enable the spores to germinate in more acid solutions than in the first series, for a few spores germinated in a solution at  $P_H$  3.6. Within the same ranges, also, more presoaked spores germinated than spores which were sown directly on the phosphate solutions.

TABLE V.—The effect of hydrogen-ion concentration on the germination of spores of *Urocystis tritici* sown on phosphate solutions (A) and presoaked in distilled water before transfer to phosphate solutions (B)

$P_H$ .	Percentage of germination.		$P_H$ .	Percentage of germination.	
	A.	B.		A.	B.
3.2	0	0	5.4	27	78
3.2	0	0	5.7	38	82
3.6	0	<sup>a</sup> Trace.	5.7	45	71
3.6	0	<sup>a</sup> Trace.	6.4	14	12
4.1	6	22	6.4	11	16
4.1	3	34	7.1	<sup>a</sup> Trace.	0
5.1	40	92	7.1	<sup>a</sup> Trace.	0
5.1	32	85	8.3	0	0
5.4	30	68	8.3	0	0

<sup>a</sup> Less than 1 per cent; no sporidia formed.

The optimum concentration for germination appeared to be between  $P_H$  5.1 and 5.7. In those concentrations in which only a few spores germinated—namely,  $P_H$  3.6 and  $P_H$  7.1—no sporidia were produced.

These results correspond fairly closely to those which had been obtained previously when small quantities of young wheat-plant tissue were added to the solutions, although in these studies the procedure enabled the spores to germinate in solutions which were originally slightly more alkaline than those in which germination occurred in the present experiment.

The previous treatment of the spores is thus an important factor in determining their subsequent behavior at germination. Hence, it might be expected that previous exposure to other environmental conditions might also have an effect on the nature and extent of germination in solutions of different hydrogen-ion concentration.

The above results, however, indicate a set of conditions within which the spores of *Urocystis tritici* might be expected to germinate readily. There apparently is no information as to the behavior of the spores in relation to various conditions of soil acidity. Laboratory experiments thus merely indicate that a somewhat similar relationship might be expected to occur in the field. Other conditions being favorable, the spores germinate most readily when in contact with slightly acid solutions.

#### VIABILITY OF SPORES

It is not definitely known how long the spores of *Urocystis tritici* may retain their viability in the soil, although there is evidence that some spores may live for a considerable number of years. McDiarmid (30) reports a case of infection in a crop after an interval of seven years. The seed was treated with formaldehyde solution and was sown on land which seven years previously had borne a diseased crop, but which had not been cropped since that time. Such evidence, of course, is far from conclusive, since there is a possibility that spores may have been blown on to the land in the meantime. McAlpine (29) reports some pot experiments in which he was unable to secure infection with spores that were more than 12 months old, although he does not consider these results as entirely conclusive.

In experiments made by the writer it appeared that very few spores were viable after they had been kept 28 months in the laboratory. Repeated tests were made with spores which had been kept in the laboratory for periods ranging from three to nine years, but in no instance did any of the spores germinate. On the other hand, there is a general impression that the spores will retain their viability for several years. It thus appears that environmental conditions may have a very marked influence on the viability of the spores.

Several investigators have studied the effect of controlled temperature and moisture conditions on the viability of the spores of a number of plant pathogenes. Recently Peltier (37) has observed that the viability of the urediniospores of *Puccinia graminis tritici* Form III is profoundly affected by the relative humidity and the temperature to which the spores are exposed. He found that the spores retained their viability longest within a range of medium relative humidities.

In experiments on the viability of the spores of *Urocystis tritici*, the writer has used spores of different ages and from different sources, under controlled conditions of temperature and relative humidity. Relative

humidities of 0, 10, 25, 50, 75, 90, and 100 per cent were secured by the use of sulphuric acid in a series of dilutions, according to the method described by Wilson (56). The solutions were placed in wide-mouthed bottles. Infected leaves were cut into sections about 5 mm. in length, and were then suspended over the solutions in small paraffined containers fastened to the undersurface of the corks, the latter being sealed down with paraffin. The bottles were placed in incubators at the following temperatures: 5° to 7° C., 18° to 21°, 26.5° and 37°. The results of early experiments are not recorded, as the spores did not germinate readily in any known nutrient solution. The spore material used in the following experiment had been kept for eight months in the laboratory prior to the commencement of the experiment. Germination tests were made at approximately monthly intervals over a period of five months and, on each occasion, small portions of wheat-seedling tissue were used to stimulate the germination of the spores in water by the method already described.

Under the conditions of the experiment there was no marked difference in the amount of germination at each relative humidity within a temperature range of 5° to 26.5° C. At 90 and 100 per cent relative humidities, however, it became difficult to separate the spores from the host tissue. Consequently, there was a greater amount of contamination in these series and the results are not entirely comparable with those obtained in the lower relative humidities. It was observed also that, even after the tissue had been exposed to 100 per cent relative humidity for five months at the temperatures ranging from 5° to 22°, sometimes almost all the spores of certain small batches germinated.

The consolidated figures for the percentage of germination at all relative humidities for each of the three lower temperature ranges (5° to 26.5° C.) indicate a definite relationship between relative humidity and the viability of the spores (Table VI). Seventy-five per cent relative humidity appeared to be the optimum for the retention of viability at these temperatures. The figures obtained for the 50 per cent relative-humidity series indicate that this also is very favorable for the retention of viability. At both higher and lower humidities, comparatively few spores germinated toward the end of the experiment. This was especially true in all cases at the lower humidities under the conditions of the experiment.

TABLE VI.—The effect of humidity on the viability of spores of *Urocystis tritici* eight months old at commencement of experiment on November 22

Approximate relative humidity.	Relative germination on— <sup>a</sup>						Totals for each relative humidity.
	Dec. 13.	Dec. 29.	Jan. 31.	Feb. 27.	Mar. 25.	Apr. 25.	
0.....	86	85	16	4	3	8	202
10.....	68	152	22	11	7	33	293
25.....	6	131	75	105	22	55	394
50.....	192	177	196	236	149	148	1,098
75.....	261	208	207	243	202	185	1,306
90.....	157	25	51	6	63	68	370
100.....	114	158	43	5	31	6	357
Totals for all temperatures and all humidities.....	884	936	610	610	477	503	.....

<sup>a</sup> Totals of percentage of germination at each relative humidity for three temperature series (5° to 26.5° C.).

A consideration of the totals for all temperatures and all humidities shows, on the whole, a tendency toward progressive decrease in the relative number of spores which germinated, but at 75 per cent relative humidity there was apparently no significant decrease in the amount of germination during the experiment.

In the 37° C. series, a few spores germinated within a range of from 0 to 50 per cent relative humidity after exposure for five months from the commencement of the experiment.

When tissue containing fresh spores was exposed to the relative humidity series, it was found that drying over concentrated sulphuric acid at first accelerated the germination of a large number of spores, but constant exposure to this condition eventually inhibited germination. Spores exposed to the medium relative humidities reached their maximum germination capacity later than did those in the low relative humidities, but this capacity for germination was retained for over six months in a manner similar to that observed in Table VI.

It was observed also that the spores from each of the three lower temperature ranges (5° to 26.5° C., inclusive), which had been kept for several months in the 50 to 75 per cent relative humidity series, frequently began to germinate three days after their transference to distilled water at 22°. Plant tissue subsequently was added to the water containing these spores just as in the other instances in order to obtain comparative readings throughout. However, it appears that exposure of the spores to the above-mentioned relative humidities causes a change in their physiological constitution, which is apparently similar in some respects to that induced by the various stimulatory agents described above.

It is obvious that a number of limitations are to be noted in the experiments just described. It may well be expected that there are differences in the maturity of spores in the various leaf sections, and possibly also inherent individual differences in spores which are considered equally mature, for spores produced under varying environmental conditions may respond differently to the influence of the controlled environmental conditions of such an experiment. The question arises, also, as to whether the undisturbed spores of the sorus are completely affected by the relative humidity to which the tissue is subjected. Exposed spores undoubtedly would be more readily subject to this influence, although under natural conditions they often may remain in the tissue for considerable periods. In making the germination tests, it evidently is not practicable to maintain absolutely uniform conditions throughout. However, it is believed that a consideration of the consolidated totals of percentage germination at each relative humidity indicates that, although the extreme limits have not yet been determined, medium relative humidities favor the longevity of the spores.

## INOCULATION AND INFECTION

### SEEDLING INFECTION

McAlpine (29) first reported inoculation experiments with this fungus. Infection occurred when spores were dusted on the seed and also when clean seed was sown in infested soil. There is no available information, however, as to the actual conditions under which infection may occur. Reference frequently has been made to the fact that seedlings are susceptible to certain smut fungi only during a very limited period.



The stage of growth of the host at this period is of great importance. McAlpine (29) also has pointed out that environmental conditions which favor a prolongation of this critical period are generally conducive to heavier infections. In discussing resistance to bunt, he mentions that differences in susceptibility of the wheat varieties may sometimes be correlated with differences in rapidity of their seedling growth.

In preliminary experiments with dry spores of *Urocystis tritici*, the writer inoculated wheat seedlings of several varieties of wheat known to be susceptible to flag smut (Federation, Marshall's No. 3, and Canberra) and sowed them in moist soil at from 18° to 23° C., but in no instance was infection noted when the coleoptiles were more than 4 mm. in length at inoculation.

As a result of subsequent investigations, it is highly probable that seedlings in later stages of growth may become infected when inoculated with germinating spores and kept in a suitable environment.

#### AMOUNT OF INOCULUM

Several investigators have called attention to the relationship between the amount of inoculum employed and the subsequent development of the disease. In this connection, McAlpine (29) has recorded the results of inoculation experiments with bunt spores. When inoculum was applied at the rate of 1 bunt ball per 5 kernels, there was from 79 to 81 per cent of infection; when applied at the rate of 1 ball per 100 grains, there was from 56 to 58 per cent of infection.

Heald (15) has indicated a quantitative relationship between the spore load in seed wheat and the percentage of stinking smut produced in the crop. Using artificially smutted seed, a load of 36,000 to 150,000 spores of *Tilletia tritici* was necessary to produce maximum infection. He suggests that either multiple infection occurs or that there is a chemical mass effect due to the number of spores.

There are some points of similarity between infection of the wheat seedling by the flag smut organism and infection by the bunt organism. Infection may result from inoculum on the seed or from inoculum present in the soil. The germ tubes or infection hyphae penetrate the tissues of the coleoptile and establish parasitic relationships within the young growing tissues of the host.

In pathogenicity studies with *Urocystis tritici*, infection frequently has been difficult to secure by means of dusting the spores on the grain, even though large amounts of inoculum were used, but when the young coleoptiles were inoculated with several platinum loopsful of germinating spores and the seedlings subsequently incubated under favorable conditions, almost complete infection resulted. Hence, a few spores germinating under suitable environmental conditions may be as effective in producing heavy infection as a much larger number in which the percentage of germination is relatively low.

In flag-smut pathogenicity experiments in the past, dry spores have always been used as a source of inoculum on account of the very capricious germination of spores. In laboratory tests it has been difficult to germinate the spores, but they appeared to germinate somewhat more readily under certain conditions existing in the soil. At other times, however, it appeared as if the spores did not germinate readily even under apparently favorable soil conditions. This fact seems to constitute one of the reasons why the flag-smut organism is such an important pathogene

under Australian conditions. If the spores germinated readily, it would be a simpler matter to free the soil of viable inoculum merely by rotation of crops, and control would be largely a question of seed disinfection. At present, however, under Australian conditions, the inoculum in the soil appears to be the major source of infection.

#### RELATION TO MOISTURE

It is generally conceded in cases of soil infestation by cereal smut organisms, other factors being favorable, that there is a fairly close correlation between the amount of smut in the crop and the amount of moisture present in the soil during the period prior to sowing.

McAlpine (29), in flag-smut infection experiments, reports that sowings made in dry, infested soil resulted in 14 per cent of infection when germination of seed of wheat and fungous spores occurred simultaneously, immediately following the first rains. When sowings were made on adjacent plots one month after rain, only 1 per cent of the plants became infected.

Heald and Woolman (16), in discussing the relation of moisture to infection of wheat by bunt, state that sowing in dry soil and waiting for rain is better than sowing in a very wet soil. Mackie (31) also observed that there may be sufficient moisture in a soil for germination of wheat seed and yet be insufficient for germination of the bunt spores. Hungerford (20), in studies on the same disease, reports that there is a very definite relationship between the amount of moisture at seeding time and the amount of bunt in the resulting crop of wheat. A high soil moisture content at planting was conducive to heavy infection. These authors agree that very little infection results if sowing is delayed for some weeks after the incidence of heavy rains. The spores of *Tilletia tritici* rapidly lose their power to infect, particularly if the soil is cultivated frequently.

Jones (22) records that the spores of *Ustilago avenae* (Pers.) Jens. do not germinate readily in very moist soils. She states, "Germination percentages were greatly reduced at 80 per cent of the water-holding capacity."

Walker and Jones (52) have shown that soil moisture is not a limiting factor in the development of the onion-smut fungus, *Urocystis cepulae* Frost. They state that "a high percentage of infected plants resulted over the entire range in which good germination and growth of the host occurred."

In greenhouse experiments with spores of *Urocystis tritici*, the writer has observed that wheat plants may become infected when seed is sown in infested soil which had been watered constantly for five weeks. The writer has shown in a previous paper (35) that the moist spores rapidly lose their viability when subjected to a temperature of 27.5° C. for a few hours. Spores which had been presoaked in distilled water for five days at 20° failed to germinate after exposure for 36 hours at 27.5°. There are thus two possible reasons for reduction of amount of viable inoculum in moist soil: (1) The spores may germinate and perish in the absence of a suitable host and (2) they may lose their capacity for germination.

It has been mentioned above that the spores of *Urocystis tritici* will produce sporidia either in moist air or when totally submerged in water. It is possible that there are certain limits of soil moisture

content within which germination of the spores may occur most readily. However, the fact that the spores can germinate and produce sporidia when entirely submerged in water would indicate that there is not necessarily any upper limit such as has been indicated for the oat-smut organism described by Jones (22). Hence, a special study was not made of this factor under soil conditions.

#### RELATION TO TEMPERATURE

The writer (35) has stated elsewhere that the optimum temperature for germination of the spores of *Urocystis tritici* is between 18 and 24° C. It was observed that spores presoaked in water for six days at 20° germinated most readily at 24°, but that spores exposed to constant temperature only, germinated most readily at 18°.

From field observations and the results of laboratory studies it appeared that soil temperatures might have an important influence on the development of flag smut. The following experiments, therefore, were made in a series of soil-temperature tanks.

The containers were of galvanized iron, about 18 inches deep and 8 inches in diameter. Each held approximately 17 kgm. of clay-loam soil, the moisture content of which had been adjusted to 17.5 per cent on a dry-soil basis. The surface of the soil was about 1 inch below the level of the water in the tank, thus making it possible to maintain a fairly uniform temperature throughout the container.

After the first adjustment for moisture, water was applied through a central tube, which extended to a small crock at the base of the container. The soil was not sterilized, because it was known that it did not contain spores of the flag smut organism. A range of four temperatures was used in each series. By means of suitable adjustment, it was found possible to maintain the series fairly constantly within the following ranges: 14° to 15° C., 19° to 21°, 24° to 26°, and 29° to 31°. Although the air temperature of the above-ground portions of the plant ranged from 15° to 29°, it remained fairly constant at 22°. Thus the soil temperature could have only a direct effect on the process of entrance and infection, although it is possible that there also may be an indirect effect on the parasite due to changes in the physiological condition of the host induced by high soil temperatures.

Canberra, an Australian wheat susceptible to flag smut, was used throughout the test. The seed was treated three minutes in a 1½ per cent solution of copper sulphate and, because of the low percentage of germination, only germinating seeds were planted. From 20 to 24 such seedlings were sown in each container at a depth of from one-half to three-quarters of an inch. Four containers were used at each temperature, a single container being used for each test. The spores used for inoculation were approximately six months old and were known to germinate promptly in laboratory tests.

In the first series (pots 1, 5, 9, and 13), the seedlings were inoculated with spores which had been presoaked on the surface of distilled water for three days at 20° C. Pots 2, 6, 10, and 14 contained seedlings inoculated with dry spores. Spores which had commenced to germinate were used to inoculate the seedlings in pots 3, 7, 11, and 15. In each of these instances the seedlings were inoculated and then sown immediately at the respective temperatures. The final series (pots 4, 8, 12, and 16) contained seedlings inoculated with germinating spores and sown at 15°.

Six days later they were transplanted to the containers in the temperature series. At this time the first leaf of each plant was approximately 3 inches long.

After the seedlings were planted, waxed paper and cotton wool were placed over the surface of the soil to minimize evaporation until the plants had appeared above the soil. Although it was not possible to maintain equivalent and constant moisture conditions throughout the experiment, it is probable that the moisture content of the soil in all of the containers was practically the same until just after infection had occurred. During the course of the experiment, the containers were weighed once a day and water was added to replace the losses from evaporation and transpiration. The moisture content throughout appeared to be adequate for the growth of the host plants.

The seedlings were planted on January 18 and final observations were made on April 3, as by this date all uninfected plants in the three lower-temperature tanks had headed. The plants kept at from 29° to 31° C. were removed from the influence of high temperature on this date, but no smut developed subsequently. The results of the inoculations are given in Table VII, and the condition of the plants when approximately 10 weeks old is shown in Plate 2. In estimating the percentage of smutted plants, all that were smutted or partly smutted are included.

The disease appeared in plants of various ages. The first lesions were observed on two plants in pot 8 and in one in pot 12, 29 days after inoculation (Pl. I, A). The fifth leaf of both plants was heavily infected. It is observed that all of these plants were inoculated at 15° C. and were subsequently transferred to higher temperatures. Other plants were observed in which the fourth leaf contained typical lesions of the disease. The lesions on these plants were evident 32 days after inoculation.

In contrast to the above, no signs of disease appeared on several plants in pot 1 until 84 days after inoculation.

TABLE VII.—*The effect of soil temperature on the development of Urocystis tritici in seedlings of Canberra wheat*

Pot No.	Temperature.	Inoculum.	Amount infection. <sup>a</sup>	Per cent infection.	Remarks.
	°C.				
1	14-16.....	Presoaked spores.....	$\frac{1}{2}$	54.5	6 plants partially smutted; 2 plants smutted at heading.
2	.....do.....	Dry spores.....	$\frac{1}{2}$	71.4	9 plants partially smutted.
3	.....do.....	Germinating spores....	$\frac{1}{2}$	88.9	3 plants partially smutted.
<sup>b</sup> 4	.....do.....	.....do.....	$\frac{1}{2}$	80.0	2 plants partially smutted.
5	19-21.....	Presoaked spores.....	$\frac{1}{2}$	66.7	6 plants partially smutted.
6	.....do.....	Dry spores.....	$\frac{1}{2}$	52.2	4 plants partially smutted.
7	.....do.....	Germinating spores....	$\frac{1}{2}$	84.2	3 plants partially smutted.
<sup>b</sup> 8	.....do.....	.....do.....	$\frac{1}{2}$	93.8	1 plant partially smutted.

<sup>a</sup> Numerator=number of plants infected; denominator=total number of plants.

<sup>b</sup> Seedlings transferred to temperature series after inoculation and six days' growth at 15° C.

TABLE VII.—The effect of soil temperature on the development of *Urocystis tritici* in seedlings of Canberra wheat—Continued

Pot No.	Temperature.	Inoculum.	Amount infection. <sup>a</sup>	Per cent infection.	Remarks.
	°C.				
9	24-26	Presoaked spores	$\frac{3}{17}$	17.6	2 plants partially smutted.
10	do	Dry spores	$\frac{2}{21}$	9.5	
11	do	Germinating spores	$\frac{5}{17}$	29.4	1 plant partially smutted.
b 12	do	do	$\frac{9}{16}$	56.3	
13	29-31	Presoaked spores	$\frac{0}{14}$	0	
14	do	Dry spores	$\frac{0}{13}$	0	
15	do	Germinating spores	$\frac{0}{21}$	0	
b 16	do	do	$\frac{1}{13}$	7.7	

<sup>a</sup> Numerator=number of plants infected; denominator=total number of plants.<sup>b</sup> Seedlings transferred to temperature series after inoculation and six days' growth at 15° C.

Occasionally signs of disease were visible on younger tiller shoots before any lesions appeared on the main shoot. Instances occurred on two plants in the 14° to 16° C. series in pot 1, in which presoaked spores had been used as inoculum.

In considering the effect of temperature on the development of the disease, it would appear from Table VII that the heaviest infection occurred in the series kept at from 14° to 16° C. and 19° to 21°. This is apparent whether the inoculations are made with ungerminated or with germinated spores. On this basis alone, there are no significant differences between the results from either of these series. However, judged on the date of the first appearance of the disease and the degree of infection produced in the plant, the series at 19° to 21° more closely approaches the optimum conditions for infection and subsequent development of the smut (Pl. 2).

The most severe infections recorded in the experiment occurred in pots 7 and 8. In these cases, germinating spores were used as inoculum. Practically all the plants were completely affected and the disease appeared in all such plants approximately six weeks from date of inoculation. Plants inoculated with germinating spores consistently became more heavily infected than those inoculated with ungerminated spores.

In the lower temperature series there was a higher percentage of partially smutted plants and in the majority of instances the first appearance of the disease was considerably later than that recorded for the series at 19° to 21° C.

An effort was made to determine if inoculation at low temperature and subsequent transference of the inoculated plants to higher temperatures would favor the development of the disease. The plants in pots 4, 8, 12, and 16 were inoculated at 15° C. and then transferred to various temperature tanks, as previously described. There apparently are no significant differences between the amount of infection at 14° to 16° and at 19° to 21°, although the highest recorded infection, 93 per cent in pot 8, and the earliest recorded infections, two plants in pot 8 and one plant in pot 12, occurred in the transferred plants.

In the higher temperature series, the transferred plants were much more heavily infected than the plants which had been exposed to constant temperature throughout. In the series at 29° to 31° C., the only

infected plant recorded is one which had been transferred. It is to be remarked that, although the percentage of infection is higher in the plants transferred to high temperatures than in those exposed constantly to high temperatures, the number of infected plants is much lower than in those instances in which transfers were made to the lower temperature series.

A histological study of infected seedlings has shown that a period of six days at 15° C. is longer than is required for entrance of the parasite under the conditions mentioned above; hence, it is highly probable that the germ tubes had entered and became established in the plants before they were transplanted. The subsequent decrease in the percentage of recorded infection is due either to the direct action of temperature or to changed physiologic conditions in the host plant.

Walker and Jones (52), in discussing the effect of temperature on the development of onion smut, report that "exposure of onion plants bearing incipient infections to a temperature of from 30° to 33° C. for from 12 to 15 days almost entirely checked further development of the parasite." High air temperatures alone were insufficient to check development of the disease, and it was suggested that the inhibitory effect noted above "may be due in part at least to the influence of the environmental conditions upon the metabolism of the host and not entirely to a direct effect upon the fungus itself."

In the experiments with flag smut, the roots and the small basal portion of the shoot only were exposed to the constant temperature ranges. There is no direct evidence that a change in physiological condition of the host was responsible for checking the amount of infection at the higher temperatures, for it is possible that, as the young tissues of central portions of the plant must have been influenced directly by temperature, the fungus also must have been subject to the same influence. Laboratory studies have shown that constant exposure to temperatures above 24° C. is unfavorable to the development of the fungus, and it would appear that in pots 12 and 16 the plants were more tolerant of the higher temperatures than was the parasite.

Under the conditions of the experiment, there appeared to be no significant differences between presoaked and dry spores when used as inoculum. In these cases the only evidence as to the degree of penetration of the fungus is that derived from a consideration of the degree of infection. It appeared that in the majority of instances in which ungerminated spores were used for inoculation at low temperatures, the organism was less successful in reaching the tissues in the region of the growing point, and, in a number of instances, the main shoots escaped entirely. It appeared that the organism persisted in the lower nodes and thus was readily able to infect the young shoots which arose from them.

Previous experiments have shown that the spores will germinate at 5° C. A test was made to determine if infection might occur at this temperature. Twenty seedlings of Canberra wheat, in which the coleoptiles had just appeared, were kept at 5° for several hours, then heavily inoculated with germinating spores of *Urocystis tritici*. They were then kept in a moist atmosphere at 5° for seven days after which they were removed, thoroughly washed, heavily dusted with copper carbonate, and then planted in sterilized soil. Six weeks later 5 per cent of the plants had produced small culms which bore characteristic lesions of flag smut. None of the plants showed any sign of infection on the main stem. They were allowed to head, but no further sign of the disease appeared.

## DISTRIBUTION OF THE PATHOGENE WITHIN THE HOST

The distribution of the pathogene within the host has been studied by investigators of other smuts.

McAlpine (29) cites an instance in which a barley plant produced heads affected with loose smut, *Ustilago nuda* (Jens.) Kell. and Sw., early in the season. These heads were cut back and a second series of culms was produced. All of these were infected.

Potter (38) also has discussed the phenomenon of infection of the nodal branches of sorghum by *Sorosporium reilianum* (Kuehn) McAlp. He examined the buds on individual culms for presence of the parasite. In some cases the basal portion of the culm escaped, and occasionally the top grew away from the parasite, although, in this instance, it usually remained sterile. The regularities in infection of these buds indicated that they were infected early, rather than that primary infection occurred. Potter states that the extent to which the mycelium develops during the first few weeks while the sorghum plant is growing slowly determines the final extent of infection.

Histological studies of the tissues of plants infected with flag smut indicate that the mycelium is frequently observed at the nodes even when the main shoot is of considerable length (fig. 1, d, and Pl. 3, D, a).

In tests on partially infected plants in the soil-temperature series, all infected culms were removed, and it was observed that the new culms which subsequently developed were most frequently infected. In other instances infected shoots were observed to arise from nodes almost 2 inches from the level of the ground and on main stems otherwise showing no sign of infection.

From the above it would appear that those conditions which favor the development of secondary culms might very readily result in an increase in the amount of damage resulting from flag smut.

It is possible that the relatively crowded condition of the plants in the soil temperature containers was sometimes unfavorable to the production of tillers. Several plants, consisting of a single stem only, produced normal heads and thus did not have as full an opportunity of indicating the presence of the parasite as did those plants which produced secondary shoots.

Hecke (17) has described a form of infection which he terms "Triebinfektion." He claims to have produced infection of the young shoots of *Melandryum* by cutting back 2-year-old plants and dusting the crown with spores of *Ustilago violacea* (Pers.) Fuckel. Subsequent shoots were smutted. A similar experiment was conducted with the perennial rye, *Secale montanum*. The plants were cut back to the crown and dusted with spores of *Urocystis occulta* (Wal.) Rab. The shoots which subsequently developed were affected with smut. Hecke suggests that this mode of infection is not restricted to these organisms but that it occurs with other smut fungi.

An unsuccessful attempt was made by the writer to produce flag smut in wheat by a similar method. Twenty-four plants of Federation wheat were cut back to within one-fourth of an inch of the ground level, the soil was removed from the crown and the upper roots, and the plants were heavily inoculated with spores of *Urocystis tritici*. Soil then was added to cover the plants completely, but the smut did not appear in any of the young shoots which subsequently developed. It is possible that the results were negative because few spores germinated. More

conclusive proof, however, might be obtained by inoculating very young shoots with large numbers of germinating spores. This has not yet been attempted.

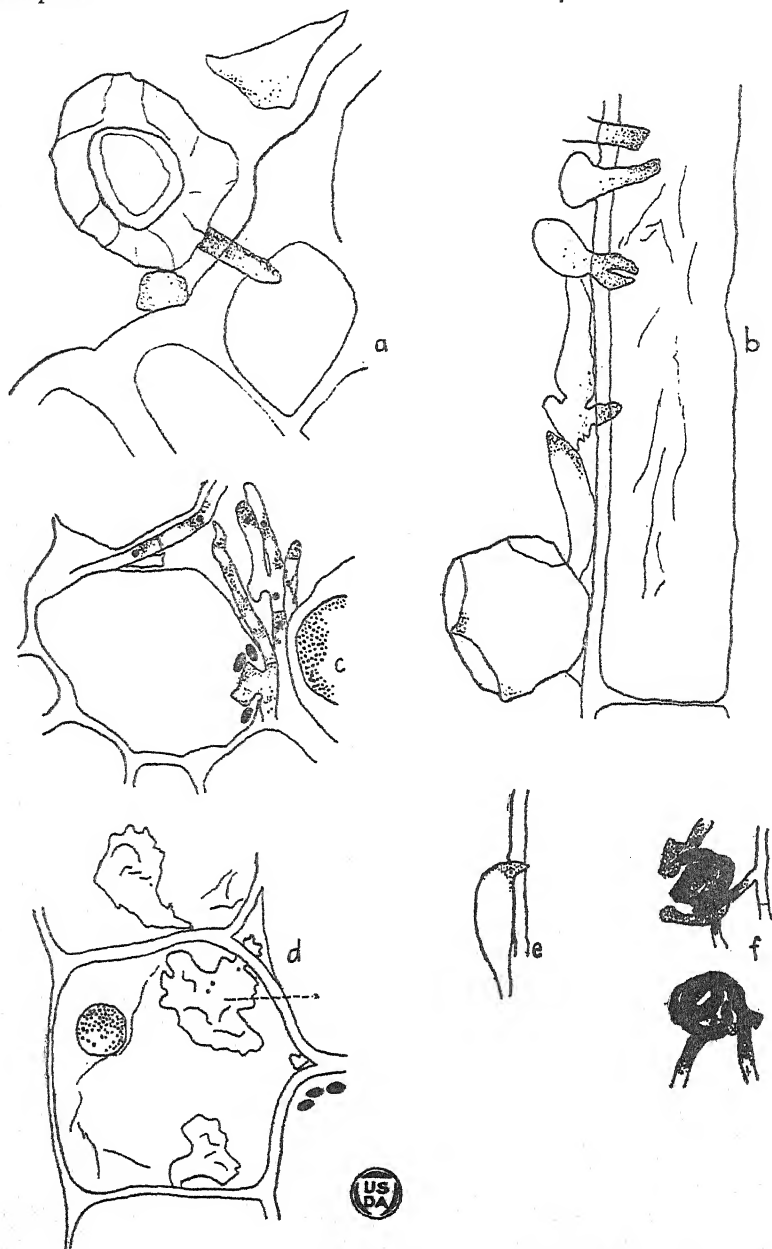


FIG. 1.—Mycelium of *Urocystis tritici*: (a) Penetration of epidermis of coleoptile of wheat seedling; (b) penetration of epidermis of coleoptile of rye seedling; (c) intercellular mycelium in parenchymatous tissues of the leaf; (d) intracellular mycelium (X) in tissues of nodes of infected wheat plant; (e) appresorium-like structure formed by infection hypha on surface of coleoptile; (f) early stage of spore-ball formation. Camera lucida drawings (X 900).



Although the possibility of "Triebinfektion" is admitted, it is felt that such a source of infection of secondary shoots probably is of less frequent occurrence than infection of such shoots by mycelium already present in the plant.

#### DEVELOPMENT OF THE ORGANISM AND ITS RELATION TO THE HOST

##### INTRODUCTION

A study of the germination of the spores of *Urocystis tritici* has indicated that there may be considerable variation in the morphological character of the promycelium and of the sporidia. It is an interesting fact that sporidia are formed not only in air but also within a liquid substrate. This rather suggests that the physiological constitution of these bodies is different from those produced by most members of the Tilletiaceae, for it usually is considered that the members of this family produce sporidia only in air.

Brefeld (3, p. 175, 212, pl. 11) has discussed the morphological features of the germination of *Urocystis ranunculi*, *U. occulta* (Walr.) Rab., and *U. filipendulae* Tul. Sporidial structures are produced by these species, but he has questioned whether they should be considered as sporidia and has suggested that perhaps they are sterigmata, which no longer produce sporidia. McAlpine (29), on the other hand, states that the structures produced by the promycelium of *U. occulta* should be considered true sporidia because they germinated by putting forth a germ tube. More recently Paravicini (36) has stated views similar to those advanced by Brefeld.

The spores of *Urocystis tritici* resemble those of *U. occulta* in many respects. The character of the germination of the spores also is practically the same in each species. Hence, it was thought that a more detailed study of the germinating spores of *U. tritici*, especially with reference to the nuclear phenomena involved, might yield more exact information on the nature of the structures produced and their significance in the propagation of the organism.

A number of investigators have studied the nuclear phenomena involved in various phases of the life history of many smuts. In this connection attention was first directed to the nuclear condition in the young spore. Dangeard (10) studied the development of spores in a number of genera of the Ustilaginaceae and Tilletiaceae and found that the young spores consistently contained two nuclei which later fused. This fact has since been confirmed by Maire (33), Lutman (28), Rawitscher (41), and others.

In a discussion of some features of the germination of spores of certain of the Tilletiaceae, Dangeard (10) also stated that the nonseptate promycelium of *Urocystis violae* (Sow.) F. de W. and *Tilletia caries* Tul. contained eight nuclei derived from the single fusion nucleus, and a single nucleus then passed into each of the eight sporidia.

A similar observation has been made by Paravicini (36) for *Tilletia tritici* (Bjerk.) Wint., *Entyloma calendulae* (Oud.) de B., *Urocystis anemones* (Pers.) Wint., and *U. violae* (Sow.) F. de W. In these forms he states that typically a nucleus wanders to the end of the promycelium and divides there several times, a single nucleus then passing to each of the sporidia.

Lutman (28) studied the nuclear condition of the mycelium and nuclear phenomena at the time of spore formation in a number of smut fungi belonging to the Ustilaginaceae and to the Tilletiaceae. He states that in the Ustilaginaceae the mycelium is characteristically multinucleate, whereas the mycelium of the Tilletiaceae frequently contains binucleate cells. In the latter family, *Entyloma nymphaeae* (Cunn.) Set. had binucleated cells and this condition also was characteristic of the older mycelium of *Urocystis anemones* (Pers.) Wint., *Doassansia alismatis* (Nees) Cornu., and *D. deformans* (Setch.). Paravicini (36) studied certain members of this family and observed that the sporidia conjugated in pairs, with or without the intervention of a fusion canal, and that a nucleus wandered from one sporidium to the other and thus established a binucleate condition. In *U. anemones*, fusions were seen only rarely, and in *U. violae*, where he observed binucleate sporidia with a fusion canal between them, he was of the opinion that nuclear migration occurred between sporidia also. He states that in both families of the Ustilaginales the binucleate condition arises through conjugation of the sporidia or hyphal cells and is maintained until the spores are formed.

He states that when the spores of *Urocystis anemones* germinated, sporidia were not formed, but he describes the appearance of easily detached mycelial threads which at first contain a single nucleus. Conjugation of these bodies was not observed, but they became septate, each cell contained a single nucleus, and finally certain of these cells became binucleate as a result of the migration of a nucleus from an adjoining cell. He draws an analogy between these mycelial threads and the promycelium of such forms as *Ustilago tritici* and *U. nuda*, although a phylogenetic relationship is not suggested. Paravicini has stressed the fact that conjugation of sporidia is of common occurrence and considerable significance in the sexuality of the smuts.

Lutman (28), however, has stated that "it is probable that the parasitic mycelium rarely or never starts from the conjugated conidia or promycelial cells even though they represent the old method of reproduction."

#### NUCLEAR PHENOMENA AT SPORE GERMINATION

The nuclear phenomena of the germinating spores of *Urocystis tritici* or of *U. occulta* apparently have not been studied. There are some marked morphologic resemblances between the promycelia and sporidia in these forms and the homologous structures produced by such forms as *U. anemones*, *U. filipendulae*, and others mentioned above, but the nuclear phenomena involved at germination appear to differ from those which have been described for such forms by Paravicini.

The writer has made an extensive study of the general characters of the nuclear phenomena associated with the germination of spores of *Urocystis tritici*. Spores in different stages of germination were fixed in Flemming's weaker solution for periods up to two hours, transferred to several changes of water, and then to slides coated with egg albumen. The transfers were made with a platinum loop, this procedure being more satisfactory than direct fixation of the spores on the slide. When the slides were almost dry, a drop of 80 per cent alcohol was added as in the method described by Harper (13), and the slides were passed through the regular series of alcohols and then stained. Several staining methods

were used, but Flemming's triple stain and Heidenhain's iron-haematoxylin used alone or with orange G in clove oil counterstain gave the most satisfactory results.

Owing to the thickness of the epispore and surrounding envelope of sterile cells, the first divisions of the nucleus within the spore were not

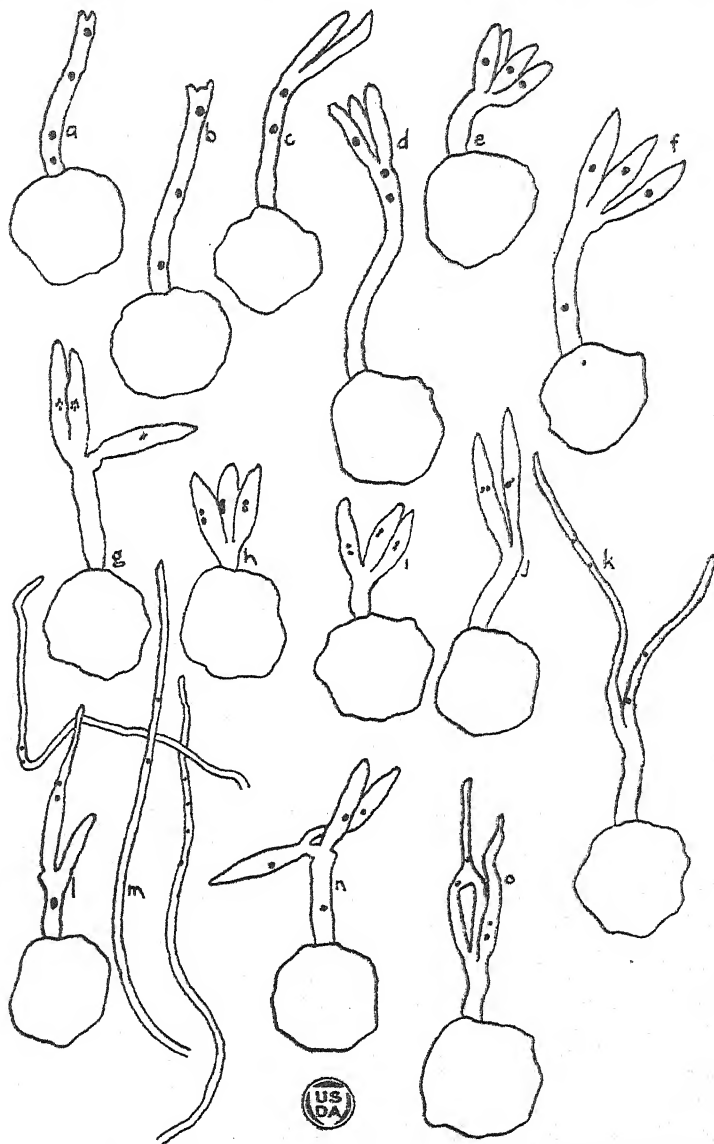


FIG. 2.—Germinating spores of *Urocystis tritici*: (a-f) migration of nuclei to sporidia; (h-j) division of sporidial nucleus; (k-l) binucleate germ tubes; (m) binucleate and 4 nucleate protoplasmic tips of germ tubes; (n-o) conjugation of sporidia. (Camera lucida drawings a-l, n-o,  $\times 200$ ; m  $\times 1,000$ .)

observed. The young promycelium, however, contained a variable number of nuclei, derived from the single nucleus of the spore (fig. 2,

a, b, c, and d). It was not apparent that a single nucleus migrated to the tip of the promycelium and divided there, but division appeared to occur in the spore and in the elongating promycelium.

As the sporidia developed, a single nucleus usually passed from the promycelium into each sporidium (fig. 2, e and f, and Pl. 3, A). Occasionally all the nuclei were not utilized in this manner, for some of them remained in the promycelium. The subsequent history of these remaining nuclei was not followed in such cases, but it is possible that they may pass into outgrowths of the promycelium which have been observed to develop after the production of sporidia.

The nuclei are small, approximately  $1.6\mu$  in diameter. At times only the nucleolus, approximately  $0.5\mu$  in diameter, was visible.

When the sporidia germinate, the single nucleus divides. Mitotic figures were not observed, although segregation of chromatin elements was apparent in several instances (fig. 2, g). The origin of a binucleate condition of the germ tube often was apparent in the sporidium before the latter had been observed to germinate (fig. 2, h, i, j, and Pl. 3, B). As germination proceeded, these nuclei were observed to have further separated until they took up positions such as are indicated in fig. 2, k and l, and Plate 3, C. In the latter instance only two nuclei are visibly in focus in the germ tube of one sporidium (Pl. 3, C, a); the other two nuclei appear as a dark band (Pl. 3, C, b). The binucleate condition of the germ tube may persist for some time (fig. 2, k, l, and m). These nuclei, however, apparently are only half the size of the original nucleus of the sporidium. Occasionally, also, germ tubes were observed in which four nuclei were present (one germ tube in fig. 2, m).

When the sporidia conjugated, it appeared as if the single nucleus of each migrated into the fusion germ tube. This, however, occurred so rarely that the complete nuclear history could not always be followed. Sometimes in a single spore some sporidia conjugated and others germinated in the manner indicated above (fig. 2, o). Each process resulted in the production of a binucleate germ tube, although the origin of the nuclei was distinct in each instance.

The process was not followed beyond the stages mentioned above. At no time, however, were the tips of the germ tubes observed to have become septate but remained unicellular. They characteristically contained two nuclei which most frequently were derived originally from the single nucleus of the sporidium.

In a histological study of the tissues of seedlings which had been inoculated with germinating spores it was found that, after infection had occurred, the infection hyphae did not contain more than two nuclei. Although the sporidia are not abstricted from the promycelium, they characteristically contain a single nucleus at first. This nucleus has been observed to divide prior to the germination of the sporidium, so that the sporidium may contain two nuclei which have arisen in a manner comparable to that in sporidia of many higher basidiomycetes. (Cf. list of number of nuclei in the cells of the Basidiomycetes, Levine, 27, p. 164-170.)

Also, the sporidia are definite structural units which germinate by means of a germ tube much smaller than the body from which it arises. Hence, it seems that they should still be considered as sporidia, although Paravicini (36) and others have suggested that homologous structures in other species of *Urocystis* should not be so considered. In some respects,

however, they differ from the sporidia produced by such genera as *Tilletia* and they present slight resemblances to an undifferentiated promycelium.

#### MYCELIUM AND SPORE FORMATION

A study of the epidermis stripped from inoculated coleoptiles indicated that entrance was chiefly effected by a germ tube which arose from a single sporidium and not from conjugated sporidia.

The mycelium is for the most part from 1.5 to 2  $\mu$  in width. In the early stages of its growth the mycelium is intracellular but it soon becomes intercellular. Its growth throughout the plant is also typically intercellular (fig. 1, c, and Pl. 3, E and G). In the older tissues—e. g., in the parenchymatous cells at the nodes of infected plants—it was observed that a large proportion of the mycelium was intracellular. The intracellular portions of the mycelium may perhaps be considered as haustoria, for they were observed to be associated frequently with intercellular mycelium, but in many cases appeared to represent a characteristic form of dormant mycelium (fig. 1, d, and Pl. 3, D, a).

In the tissues of the young leaf the mycelium was very characteristically intercellular. In heavily infected leaves the hyphae sometimes wedge the host cells apart (fig. 1, c, and Pl. 3, G). When growing rapidly, they are characteristically nonseptate, and may become branched; haustoriallike structures sometimes are observed (fig. 2, c). It is a question, however, whether these structures always represent haustoria or whether they represent mycelium which has commenced to take up an intracellular position. The mycelium varies considerably at spore formation. Those portions utilized in the formation of spores are at first narrow and nonseptate, whereas those portions not utilized become vacuolate, somewhat irregular in outline, and are most frequently septate.

The nuclear phenomena were not followed with certainty in all instances, because it was difficult to stain satisfactorily both nuclei and septa in the same mycelium. It appeared, however, that the nuclear content was not constant, one to four nuclei sometimes being observed in the cells. Hyphal fusion frequently was observed, and there were indications that nuclear migrations had occurred (fig. 1, c). The mycelium prior to spore formation, however, was fairly constantly binucleate. Fusion of hyphae was observed to occur at various stages prior to spore formation.

The method of spore formation in this genus has been studied in some detail by Wolff (57) and others. Although it appeared that the spore balls sometimes originate from a single hypha, more frequently two distinct hyphae appeared to be involved in spore formation. Branches arise which become very much coiled on one another and the spores and the spore ball envelope of sterile cells arise simultaneously in this manner (fig. 1, f, and Pl. 3, E and G).

#### RELATION TO NONSUSCEPTIBLE PLANT

While studying the relation of the fungus to a susceptible plant, studies were made concurrently on the relation of the fungus to plants resistant to flag smut. The relations between certain parasites and nonsusceptible plants have been studied by a number of investigators. Various aspects of the question of resistance and immunity have been studied in this connection, especially by investigators of the cereal rusts.

Stakman (44) has studied the action of specific forms of stem rust fungi on immune and susceptible varieties of wheat, oats, and several other hosts and has shown that there is characteristically a very definite antagonism between the protoplasmic cell contents of the immune plant and the parasite.

Woolman (58) has recently described the cytologic phenomena of infection of wheat seedlings by bunt caused by *Tilletia tritici*. He states that "*Tilletia tritici* enters the epidermis of the coleoptile of both susceptible and resistant wheat plants grown under conditions for maximum infection, but that in highly resistant varieties it develops no further. Inhibiting factors evidently are active in or just beneath the epidermis."

The writer made a histologic examination of young rye plants which had been inoculated with spores of *Urocystis tritici*. Inoculation experiments have shown repeatedly that rye is resistant to infection by *U. tritici*, but there was no information on the relationship involved. The coleoptiles of young rye seedlings were heavily inoculated with germinating spores of *U. tritici* and placed under conditions of high humidity at 17° C. The epidermis was stripped from some of the seedlings at intervals up to four days, and examined for evidence of entrance of the organism. Portions of the coleoptiles also were fixed and sectioned. A typical section is that shown in figure 2, b. It was very evident that entrance had been effected but that, owing to the unfavorable conditions within the host, development of the organism had been checked. The mode of entrance of the germ tubes resembles the mode of entrance in a susceptible host. Figure 1, e, shows an early stage of entrance of the mycelium. The germ tube swells into an appressoriumlike body and penetrates first through a small opening, somewhat similar to that figured by Waterhouse (53) for entrance of sporidial germ tubes of *Puccinia graminis* into the barberry leaf, and Leach (26) for entrance of germ tubes of *Colletotrichum lindemuthianum* into beans. No further studies were made at this time on the subsequent history of the infection hyphae of *U. tritici* within the tissues of the immune host.

#### DISCUSSION AND CONCLUSIONS

A knowledge of the reaction of a pathogene to controlled environmental conditions is fundamental to an understanding of its behavior in nature. It is well known that the severity of many outbreaks of plant disease frequently may be correlated with the environmental conditions to which the pathogene was exposed at a particular period in its life history.

Laboratory and greenhouse studies on *Urocystis tritici* have shown that the viability of the spores is profoundly affected by exposure to controlled temperatures and relative humidity. The germination of the spores, infection, and the subsequent development of the organism within the host also are greatly influenced by the conditions of the environment.

In an infested area in which it is still impracticable to replace the wheat varieties which are susceptible to flag smut by others which are resistant, adequate control of the disease may be expected only by a system of cultural practice which reduces the amount of viable inoculum, which also reduces the possibilities for infection, and finally which tends to reduce the possibilities for serious damage in the crop after infection has occurred.

The present study has furnished data which may be used as a basis for further field experimentation. Also the experimental results may be correlated in part with the present known distribution and seriousness of the disease in certain regions, and thus in conjunction with other data (distribution of wheat varieties, etc.), may serve to indicate the possibility of the disease becoming established in regions in which it does not at present occur.

#### SUMMARY

1. Flag smut of wheat, caused by *Urocystis tritici* Koern., was first found in Australia in 1868. It is now known to occur also in Japan, China, India, South Africa, southern Europe, and the United States.

2. Flag smut is one of the most destructive diseases of wheat in Australia. It is becoming more widely distributed each year and annually destroys approximately 3 per cent of the total potential wheat crop. Losses up to 70 per cent have been observed in individual fields.

3. The disease lesions may first appear on plants in any stage of growth up to heading. The earliest recorded lesions were observed on the fifth leaf of a wheat plant 29 days after inoculation. Other plants were observed in which the fourth leaf was first affected. The smut may cause considerable deformation of the host. All culms of a plant may not be affected. Under greenhouse conditions, the first formed culms of partially infected plants frequently were free from the disease.

4. The "maturation" period of the spores can be considerably shortened. Fresh spores did not germinate, although they did so after having been dried for 48 hours over concentrated sulphuric acid. Germination tests were made in the manner previously described (36).

5. Uninjured seedlings of nonsusceptible plants (field peas, beans, and rye) stimulated the germination of spores which had been presoaked in water. Such stimulation might be expected in the field when the environment causes a certain amount of anaerobic respiration of the plant roots.

6. The expressed sap of wheat seedlings at a concentration of 1 part per 10,000 was most favorable for germination of presoaked spores. The germ tubes of *Urocystis tritici* were observed to grow to a length of 4 mm. in solid media.

7. Surface films of benzaldehyde, salicylaldehyde, butyric acid, and acetone greatly stimulated the germination of presoaked spores. Other volatile substances were less effective. Benzaldehyde, 3 parts. per 2,000,000 parts of distilled water, and butyric acid, 1 part per 500,000 parts water, were suitable concentrations for such stimulation.

8. Presoaking the spores appeared to result in increased permeability of the spore envelope and thus enabled more rapid ingress of the volatile stimulant.

9. The stimulatory action recorded above was not correlated with any definite reduction of the surface tension of the medium as measured against air.

10. It is suggested that the action of the stimulatory agents is mainly such as to cause a change in the physical condition of the protoplasmic spore contents, and thus increase the permeability of the latter.

11. Preliminary tests indicate that films of some of the above-mentioned volatile materials also stimulate the germination of the teliospores of *Puccinia graminis tritici* and the spores of several other organisms.

12. According to the conditions of the experiment, the spores germinated within a hydrogen-ion range of  $P_H$  3.6 to 7.1. Optimum germination occurred within the range  $P_H$  5.1 to 5.7.

13. The relative humidity to which the spores are exposed has a marked effect on their viability. Relative humidities of from 50 to 75 per cent were most favorable for retention of viability. Spores kept at these humidities within a temperature range of from 5° to 26.5° C. frequently commenced to germinate shortly after they were placed in distilled water, without the addition of a stimulatory agent.

14. Wheat seedlings in which the coleoptiles were more than 4 mm. long did not become infected when they were inoculated with dry spores. When seedlings were inoculated with large numbers of dry spores, sometimes no infection occurred, whereas inoculations with few germinating spores at suitable temperatures consistently resulted in heavy infections.

15. Soil temperatures ranging from 14° to 21° C. were optimum for infection of wheat seedlings by *Urocystis tritici*. The most severe and earliest-recorded infection occurred on plants kept at 19° to 21°. Some plants became infected at 23° to 25°. No infection occurred at 29° to 31°. Inoculation at 15° and subsequent transfer of the plants to temperatures above 23° resulted in a decrease of the amount of infection. Some plants became infected at 5°.

16. Studies of the nuclear phenomena during germination of the spores indicate that the spore nucleus divides at germination, the nuclei migrate to the promycelium, and usually a single nucleus wanders into each sporidium. When the sporidia germinate, this nucleus divides, the germ tube becomes binucleate, and this condition may be retained for a considerable period. Germ tubes containing four nuclei were rarely observed. Conjugation of sporidia was sometimes observed. A single germ tube may be produced from the conjugated sporidia, and this germ tube at first contains the two nuclei which migrated from the sporidia.

17. The mycelium within the plant is typically intercellular, and haustoriallike bodies are sometimes observed. Branching and fusion of the mycelium occur within the plant. Prior to spore formation, binucleated hyphal cells were observed.

18. Although some spores appear to arise from a single hypha, frequently two distinct hyphae appeared to be involved. Sterile cells and spores arose simultaneously from single coiled structures, and differentiation of the cells occurred as development proceeded.

19. Infection hyphae of *Urocystis tritici* were observed to have entered the tissues of rye seedlings which are known to be immune from the disease.

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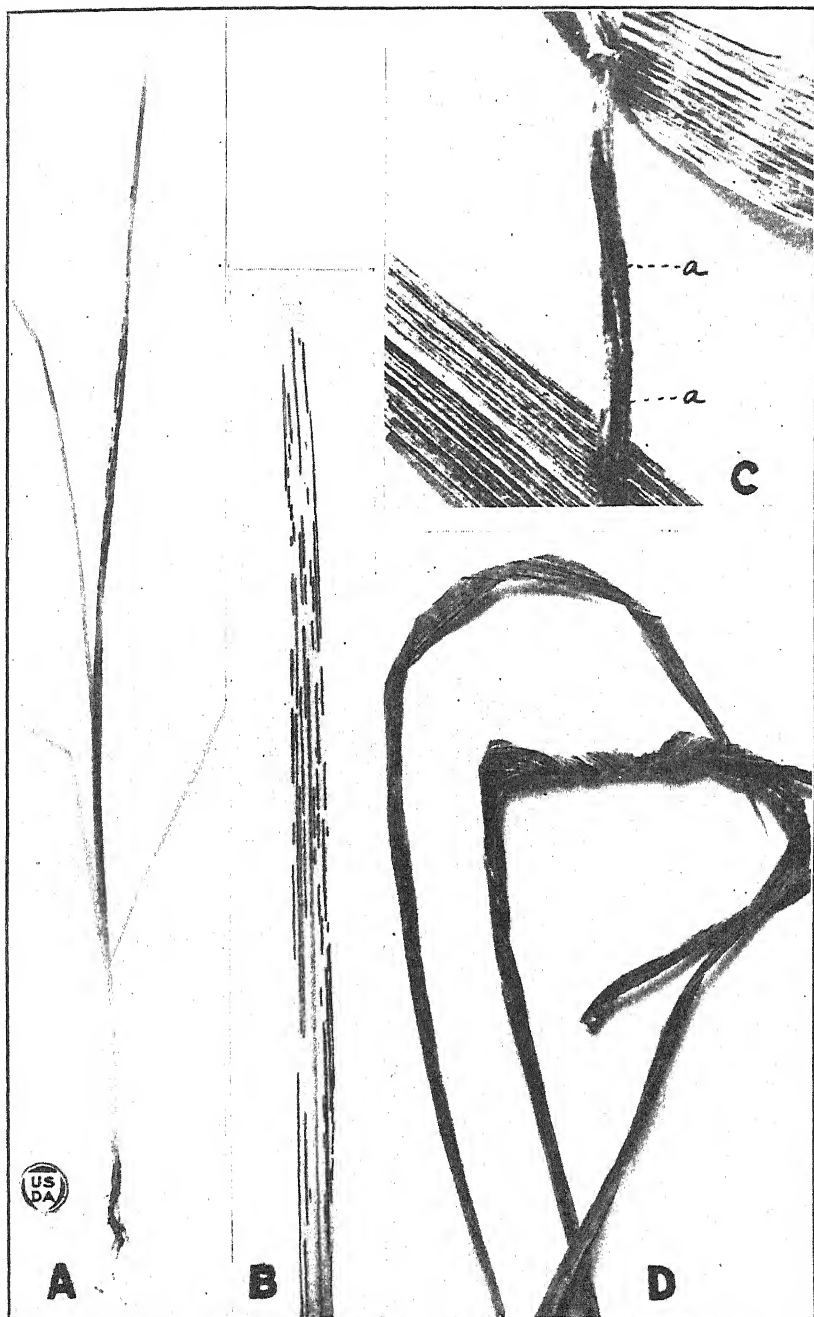
PLATE I

A.—Young wheat plant affected with flag smut, 29 days from date of inoculation.  $\times \frac{2}{3}$ .

B.—Leaf of wheat plant affected with flag smut. (Partly decolorized.) Natural size.

C.—Plant with head affected with flag smut. The darkened areas on the neck (a) represent opened sori filled with spores of *Urocystis tritici*. (Enlarged.)

D.—Portion of plant affected with flag smut showing characteristic curling and twisting of affected leaves.  $\times \frac{2}{3}$ .



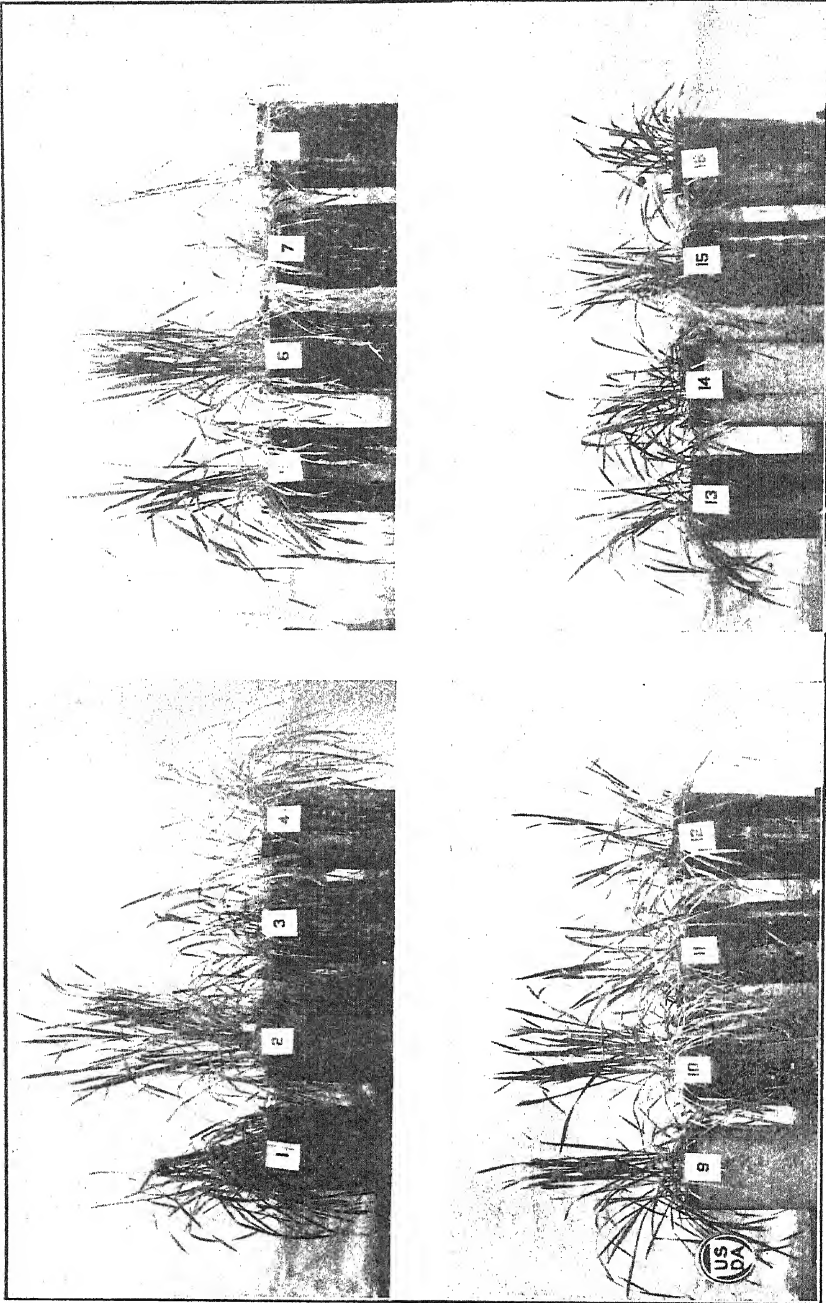


PLATE 2

The effect of soil temperature on the infection of Canberra wheat by *Urocystis tritici*:

Containers 1-4 at 14°-16° C.

5-8 at 19°-21° C.

9-12 at 24°-26° C.

13-16 at 29°-31° C.

Note destructive action of the pathogene in containers 7 and 8, in which germinating spores were used as inoculum.  $\times \frac{1}{16}$ .

PLATE 3

Germinating spores of *Urocystis tritici*, showing origin of a binucleate condition of the germ tube on germination of sporidium:

A.—Sporidium with single nucleus.  $\times 450$ .

B.—Sporidium with dividing nucleus.  $\times 450$ .

C.—Germ tube with 2 nuclei.  $\times 250$ .

Mycelium and spores of *Urocystis tritici*:

D.—Intracellular (a) and intercellular (b) mycelium at nodes of infected plants.

$\times 450$ .

E.—Intercellular mycelium, showing first stages of spore formation.  $\times 450$ .

F.—Spore formation, early stages.  $\times 450$ .

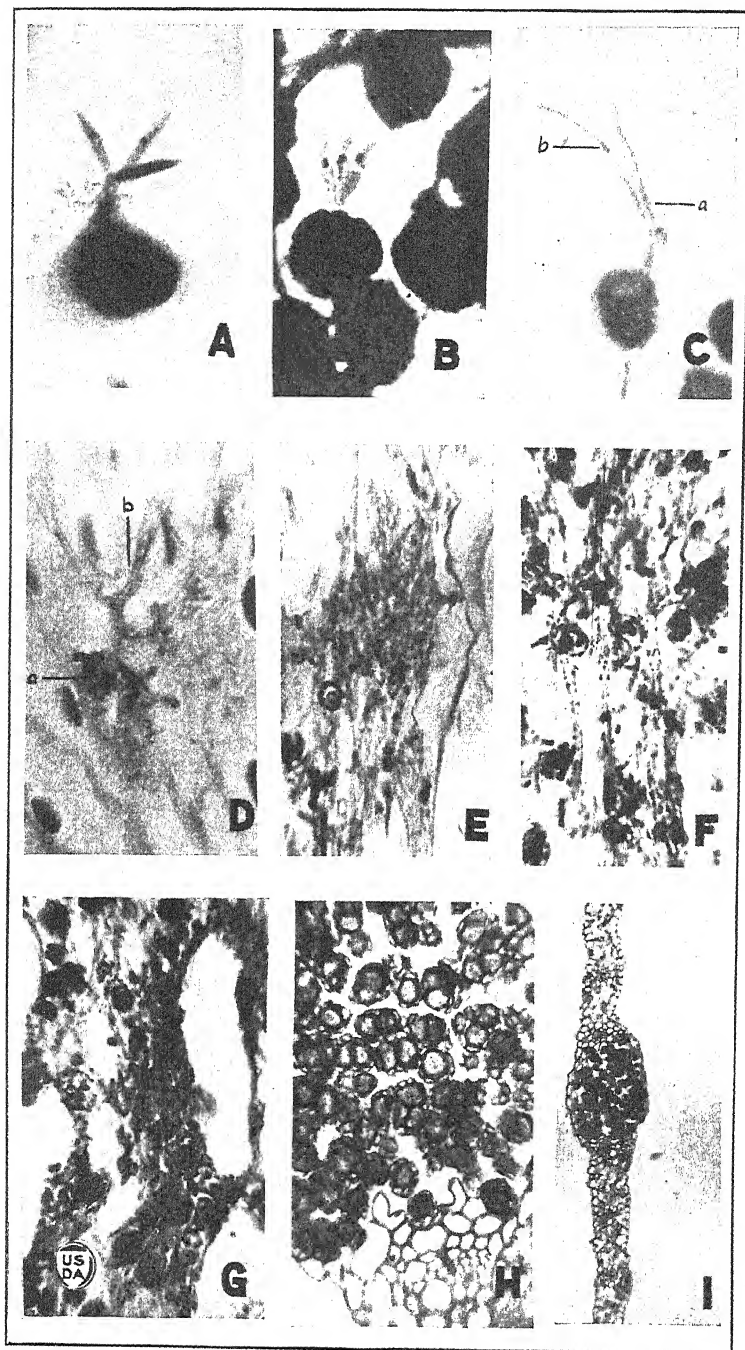
G.—Spore formation, later stages, formed for most part from intercellular mycelium.

$\times 450$ .

H.—T. S. wheat leaf showing mature spores and sporeballs in section.  $\times 200$ .

I.—T. S. wheat leaf showing sorus of spores.  $\times 40$ .







# INHERITANCE OF PETAL SPOT IN PIMA COTTON<sup>1</sup>

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## INTRODUCTION

Egyptian cotton, like the related Sea Island cotton (*Gossypium barbadense* L.) is characterized by a conspicuous red spot near the base of the otherwise yellow petal. When the flower first opens, the red pigment appears to be confined to the cells of the epidermis, but soon diffuses into the subjacent tissue. The spot is entirely lacking in most of the varieties of upland cotton (*G. hirsutum* L.).

It has been found that in a hybrid between the Pima variety of Egyptian cotton and the Holdon variety of upland cotton, the parental families having shown, respectively, pronounced development and complete absence of the spot, the spotless condition behaved as a simple recessive.<sup>2</sup> A 3:1 ratio was obtained in  $F_2$  and the behavior of  $F_3$  progenies of individuals representing different points on the  $F_2$  frequency curve confirmed the unifactorial nature of the segregation. The findings of other investigators as to the inheritance of this character are summarized in the publication referred to (p. 31).

In the Pima variety the petal spot is almost invariably well developed, although the character is rather sensitive to environmental influences and there is often variation on the individual plant in the size, shape and intensity of the spot. The normal range was far exceeded, however, by two individuals discovered in 1917 in a field of Pima cotton at Sacaton, Ariz.<sup>3</sup> The inbred descendants of these plants, closely studied during five generations, have shown at most only a very weak development of the spot while in many of the flowers the spot has appeared to be completely absent. Table I gives the mean grade of petal spot for the successive generations of these families and for the normal populations with which they were compared, the means having been based upon the averages of several flowers graded on each plant.

TABLE I.—Mean grade of petal spot in successive inbred generations of the "spotless" Pima families and in populations of this variety showing a normal development of the spot

Year.	"Spotless" families.		Normal populations.	
	Number of plants.	Mean grade of petal spot.	Number of plants.	Mean grade of petal spot.
1919.....	38	1.0 ± 0.04	13	7.5 ± 0.19
1920.....	140	.9 ± 0.04	.....	.....
1921.....	29	.9 ± 0.06	34	7.8 ± 0.04
1922.....	62	.7 ± 0.02	54	7.6 ± 0.03
1923.....	106	2.0 ± 0.04	111	8.1 ± 0.02

<sup>1</sup> Received for publication Dec. 14, 1923.

<sup>2</sup> KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 27-26, fig. 27, pl. 7. 1923.

<sup>3</sup> KEARNEY, Thomas H. HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON. In Jour. Agr. Research, v. 21, p. 239-241, pl. 54. 1921.

Isolation of these Pima families in which the spot is absent or nearly so has afforded opportunity for further study of the inheritance of the character, this time within a single variety. Crosses were made in 1920 between individuals of an inbred Pima family showing normal development of the spot and individuals of the families in which it is very weakly developed. The descendants of these crosses have been studied in the first, second, and third generations and the inheritance of the character in the intra-varietal crosses proves to be similar to that which occurred in the interspecific hybrid Holdon (upland) X Pima (Egyptian).

The heredity of the cotton plant has been studied rather extensively, but definite Mendelian segregation has been demonstrated in relatively few characters. For this reason it is advisable to give in some detail the facts as to the inheritance of petal spot, although, from the standpoint of genetics, the case presents no exceptional features. The subject has, however, a practical bearing, for the possession by an agriculturally valuable strain of Pima cotton of such a character as "spotless" petal would protect the purity of the seed by making it easy to detect the offspring of accidental cross-pollinations with other strains. Proof that this character behaves as a simple Mendelian recessive warrants the expectation that it can be transferred without great difficulty to any desirable strain of Pima cotton.

#### METHOD OF DETERMINING THE CHARACTER

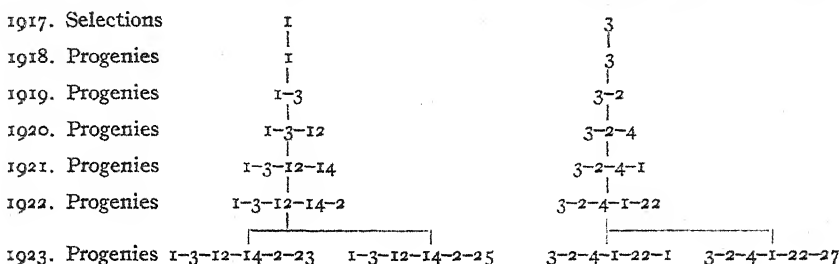
No satisfactory method for the accurate quantitative measurement of the petal spot suggested itself, so a system of grading was employed for the classification of the flowers, as in the earlier study of the upland-Egyptian hybrid. Complete absence of the spot was indicated by grade 0 and its highest development by grade 9. Grades 0, 3, 6, and 9 are represented in Plate 1. The endeavor was to have the grades indicate the total quantity of red pigment present, rather than merely the size of the area or the intensity of the color. Thus a flower having a larger but lighter colored spot might be graded the same as a flower in which the spot was smaller but more deeply colored. It is not assumed that the grading was done with perfect consistency, but it is believed that the deviation from the standard in no case exceeded one full grade and that the results obtained by this method are entirely satisfactory for the purpose of this analysis. For statistical treatment of the data, the method of grading has the advantage of making possible, in a given length of time, the classification of much greater numbers of flowers than could be handled if it were attempted to determine quantitatively the amount of pigment present.

In grading the flowers those representing the two extremes of the scale gave the most trouble. Flowers which appeared to be completely spotless when viewed with the unaided eye showed in some cases, when examined with a hand lens, very faint traces of color in the region of the spot, the red pigment being confined to scattered very small groups of cells. The practice adopted was to grade as 0 all flowers which showed no trace of red when examined in a good light without magnification. Experience indicated that the scale used should have been lengthened somewhat at the upper end, and that if the distinctions had been drawn as finely in this region as elsewhere it would have been necessary to recognize one or possibly two grades above grade 9.

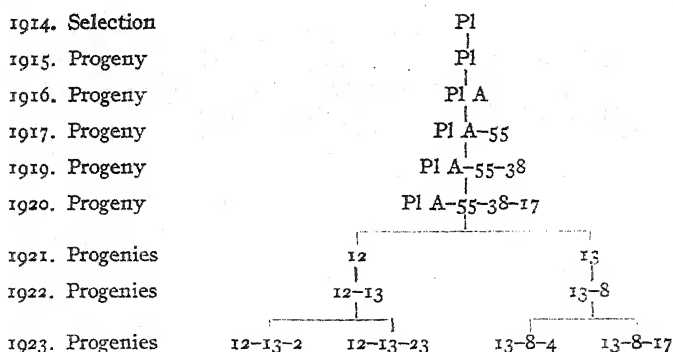
The unit upon which the frequency distributions and statistical constants are based is the average of the grades of several flowers on each individual plant. On a large majority of the plants in each generation 10 flowers were graded, and in no case is the average based upon as few as 2 flowers unless these had differed by not more than one grade.<sup>4</sup>

#### PARENTAGE OF THE CROSSES AND DESIGNATIONS OF THE POPULATIONS

The two plants selected in 1917 which became the progenitors of the "spotless" families were numbered 1 and 3, respectively. The lines of descent of the successive progenies of these individuals were as follows:



The family which furnished the normal or full-spotted parents of the crosses was descended from plant P1 of 1914, as shown in the following pedigree:



In 1920 plant No. 14 in spotless progeny 1-3-12 was crossed with plant No. 12 in normal progeny P1 A-55-38-17 and plant No. 1 in spotless progeny 3-2-4 was crossed with plant No. 13 in the same normal progeny. The spotless parents were of the second and the normal parents were of the fifth strictly inbred generation. The  $F_1$  progenies, grown in 1921, were designated 1-3-12-14  $\times$  12 and 3-2-4-1  $\times$  13. Two individuals were selfed in each of the  $F_1$  progenies, and gave rise to the following  $F_2$  progenies, which were grown in 1922:

$$\begin{array}{ll}
 (1-3-12-14 \times 12) - 20 & (3-2-4-1 \times 13) - 21 \\
 (1-3-12-14 \times 12) - 28 & (3-2-4-1 \times 13) - 24
 \end{array}$$

<sup>4</sup> As a test of the reliability of an average based upon a small number of flowers, comparison was made of the averages for the first 3 and for all 10 of the flowers graded on 100 plants in the  $F_2$  hybrid progenies. The mean departure of the 3-flower average from the 10-flower average was found to be  $0.32 \pm 0.017$  or one-third of a grade. The maximum difference, which occurred in 2 of the 100 plants, was 1.1 grade. It is concluded that averages based upon only 3 flowers would have been sufficiently accurate for most of the purposes of this investigation.

Six individuals were selfed in each of the  $F_2$  progenies and from these 24  $F_3$  progenies were grown in 1923.

In addition to the hybrid populations, progenies descended from each of the four parents of the crosses were grown each year. Endeavor was made to have the several parental and hybrid populations situated so as to eliminate environmental effect but soil heterogeneity at the Sacaton Station is so pronounced that the endeavor was not wholly successful. It is believed, however, that this factor did not impair the validity of the comparisons.

For convenience, the term "spotless" will be used henceforth to designate populations in which the spot is absent or very weakly developed. The latter condition is much the more frequent, for very few plants were found on which all of the flowers lacked even a faint trace of the spot. Populations in which the development of the spot approached the Pima norm will be designated "spotted." Strictly speaking, the allelomorphs here involved are expressed as spotless to faintly spotted, on the one hand, and full spotted on the other.

#### THE FIRST GENERATION OF THE CROSSES

Table II gives the frequency distributions and statistical constants for the parental and  $F_1$  progenies grown in 1921. For the reason given in another publication<sup>5</sup>, it is considered preferable to use the standard deviation rather than the coefficient of variation as an expression of the variability of a character determined by grading.

The data show almost complete dominance of petal spot in the first generation, although the mode of each  $F_1$  progeny is a full grade lower than that of the corresponding spotted parental population and the  $F_1$  mean is in each case significantly lower than the parental mean. The variability of  $F_1$ , as indicated by the standard deviation, is of the same order of magnitude as that of the parental populations.

TABLE II.—Frequency distributions and statistical constants, for petal spot grade, of the parental and  $F_1$  progenies grown in 1921

Population.	Number of plants.	Petal spot grade (plant averages).																	Mean.	Standard deviation.		
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0			8.5	
PARENTAL																						
12 (spotted).....	20																1	7	11	17.8±.05	0.33	
1-3-12-14 (spotless).....	12		3	2	5	2														1.2±.11	.58	
HYBRID (F <sub>1</sub> )																						
1-3-12-14×12....	28															2	14	12		7.2±.04	.30	
PARENTAL																						
13 (spotted).....	14																	5	6	3	7.9±.07	.37
3-2-4-1 (spotless).	17	1	9	5	2															.7±.06	.39	
HYBRID (F <sub>1</sub> )																						
3-2-4-1×13.....	32													1	4	14	11	2		7.1±.05	.44	

<sup>5</sup> KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID U. S. Dept. Agr. Bul. 1164, p. 13. 1923.

## THE SECOND GENERATION OF THE CROSSES

Table III gives the frequency distributions and Table IV the statistical constants of the parental and  $F_2$  hybrid populations grown in 1922. Because of the pronounced segregation in the  $F_2$  progenies, the means and standard deviations were computed separately for the spotted and the spotless class in each progeny.

TABLE III.—Frequency distributions for petal spot grade of the parental and  $F_2$  progenies grown in 1922

Population.	Number of plants.	Petal spot grade (plant averages).																		
		0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	
PARENTAL																				
12-13 (spotted).....	26																2	14	9	1
1-3-12-14-2 (spotless).....	29	1	1	5	1	3														
HYBRID (F <sub>2</sub> )																				
(1-3-12-14×12) 20.....	47	4	10	2									3	3	12	4	9			
(1-3-12-14×12) 28.....	57	1	8	5	1							1	1	3	11	10	11	5		
PARENTAL																				
13-8 (spotted).....	28																5	12	10	1
3-2-4-1-22 (spotless).....	33	1	20	12																
HYBRID (F <sub>2</sub> )																				
(3-2-4-1×13) 21.....	28	2	2	2	1							1	2	7	4	4	3			
(3-2-4-1×13) 24.....	47	1	6	3	1							1		4	9	5	7	8	2	
4 F <sub>2</sub> progenies as one array....	179	8	26	12	3							2	5	12	39	23	31	16	2	

TABLE IV.—Statistical constants for petal spot grade of the parental and  $F_2$  progenies grown in 1922, the constants being given separately for the spotted and the spotless segregates of each hybrid progeny

Population.	Class.	Number of plants.	Mean.	Standard deviation.
PARENTAL				
12-13.....	Spotted.....	26	$7.7 \pm .04$	.34
1-3-12-14-2.....	Spotless.....	29	$.7 \pm .04$	.28
HYBRID ( $F_2$ )				
(1-3-12-14×12) 20.....	Spotted.....	31	$6.7 \pm .08$	.63
Do.....	Spotless.....	16	$.4 \pm .05$	.30
(1-3-12-14×12) 28.....	Spotted.....	42	$7.0 \pm .07$	.69
Do.....	Spotless.....	15	$.7 \pm .06$	.36
PARENTAL				
13-8.....	Spotted.....	28	$7.6 \pm .05$	.39
3-2-4-1-22.....	Spotless.....	33	$.7 \pm .03$	.27
HYBRID ( $F_2$ )				
(3-2-4-1×13) 21.....	Spotted.....	21	$6.9 \pm .10$	.68
Do.....	Spotless.....	7	$.6 \pm .13$	.52
(3-2-4-1×13) 24.....	Spotted.....	36	$7.1 \pm .09$	.82
Do.....	Spotless.....	11	$.7 \pm .08$	.39

Inspection of the frequency distributions of the four  $F_2$  progenies (Table III) shows sharp segregation into a spotless and a spotted group. The percentages of spotless individuals in the  $F_2$  progenies and the departures from the 25 per cent expected with a single factor difference are stated in Table V. It is evident that the departure is significant in none of the progenies and that while the population obtained by combining the four progenies as one array shows a slight excess of spotless individuals, the departure from 25 per cent barely exceeds its probable error.

TABLE V.—Percentages of spotless individuals and departures from the expectation, in the  $F_2$  progenies grown in 1922

$F_2$ progeny.	Number of plants.	Percentage spotless. <sup>a</sup>	Departure from the expectation (25 per cent).
(1-3-12-14×12) 20.....	47	34.0	9.0±4.66
(1-3-12-14×12) 28.....	57	26.3	1.3±3.93
(3-2-4-1×13) 21.....	28	25.0	0±5.51
(3-2-4-1×13) 24.....	47	23.4	1.6±4.16
4 $F_2$ progenies as one array.....	179	27.3	2.3±2.24

<sup>a</sup> The probable error of the percentage is omitted, being the same as that of the departure from the expectation.

Reference to Table IV shows that the mean of the spotless segregates in each  $F_2$  progeny does not differ significantly from the mean of the progeny representing the corresponding spotless grandparent, except in progeny (1-3-12-14×12) 20, in which the spotless segregates gave a significantly lower mean. On the other hand, the means of the spotted segregates in  $F_2$  are in all cases significantly lower than the mean of the progeny representing the corresponding spotted grandparent and approach the means obtained in  $F_1$  (Table II). This, of course, is to be expected if dominance is incomplete, as was indicated by the results in  $F_1$ . The incompleteness of the dominance is confirmed by consideration of the variability in  $F_2$ . The spotless group in each  $F_2$  progeny is not much more variable than the corresponding spotless parental population, as is made evident by comparison of the frequency distributions (Table III) and of the standard deviations (Table IV). On the other hand, both comparisons show the spotted group in each  $F_2$  progeny to have been much more variable than the corresponding spotted parental population and the spotted groups in  $F_2$  were likewise more variable than  $F_1$  (Table II).

That the heterozygotes of the spotted group are partly distinguishable from the pure dominants is indicated by the marked bimodality of the distributions of the spotted plants in  $F_2$  progenies (1-3-12-14×12) 20 and (3-2-4-1×13) 24 (Table III). Because of the relatively small numbers involved and the nearly complete dominance of the spot, as well as the sensitiveness of this character to environmental influence, it is impossible to resolve the phenotypic ratio of approximately 3:1 into a 1:2:1 ratio. The point will be further discussed in considering the data from  $F_2$ .



TABLE VI.—Frequency distributions for petal spot grade of the parental and  $F_3$  progenies grown in 1923. The grades of the parent individuals are also given and the class of the parent, whether dominant, recessive, or heterozygous, is indicated by the letters D, R, and H<sup>a</sup>

Population	Number of plants in $F_3$	Grade of parent individual	Class of parent individual	Petal spot grade (plant averages)																				
				0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9		
PARENTAL	12-13-23.....	16	7-9	D																				
	12-13-2.....	29	7-3	D																				
	1-3-12-14-2-23.....	26	1.2	R		2	10	11	2	2	1	1												
	1-3-12-14-2-25.....			R		1	4	8	7	4	1													
HYBRID ( $F_3$ )	(1-3-12-14×12) 20-14.....	25	7-7	D																				
	(1-3-12-14×12) 20-15.....	48	7-6	D																				
	(1-3-12-14×12) 20-19.....	42	1-1	R			15	18	6	2	1													
	(1-3-12-14×12) 20-24.....	37	5-6	H			1	5	18	9	4													
	(1-3-12-14×12) 20-27.....	39	5-6	H			2	1	4	1														
	(1-3-12-14×12) 20-35.....	33	5-5	H			3	3		1														
	(1-3-12-14×12) 28-7.....	34	8-0	D																				
	(1-3-12-14×12) 28-8.....	48	8-2	D																				
	(1-3-12-14×12) 28-32.....	42	1-1	R			5	13	13	8	8													
	(1-3-12-14×12) 28-49.....	47	5-3	H			1	15	19	7	5													
PARENTAL	13-8-4.....	27	8-0	D																				
	13-8-17.....	39	7-2	D																				
	3-2-4-1-22-1.....	26	9	R			1	8	6	6	4	1												
	1-2-4-1-22-27.....	25	1-2	R			3	9	9	3	1													
HYBRID ( $F_3$ )	(3-2-4-1×13) 21-2.....	42	8-1	D																				
	(3-2-4-1×13) 21-21.....	46	7-8	D																				
	(3-2-4-1×13) 21-26.....	36	0	R			3	8	10	12	3													
	(3-2-4-1×13) 21-22.....	47	1-2	R			1	15	21	9	1													
	(3-2-4-1×13) 21-7.....	44	5-6	H			4	4	2															
	(3-2-4-1×13) 21-29.....	45	6-0	H			1	5	2															
	(3-2-4-1×13) 24-18.....	43	8-4	D																				
	(3-2-4-1×13) 24-46.....	41	8-4	D																				
	(3-2-4-1×13) 24-25.....	34	4	R			2	12	11	6	3													
	(3-2-4-1×13) 24-48.....	39	1-4	R			4	7	11	3	2													
	(3-2-4-1×13) 24-33.....	45	6-1	H			3	4	5	2														
	(3-2-4-1×13) 24-35.....	42	6-2	H			3	3	3	5														

<sup>a</sup> The table is arranged in two horizontal sections, corresponding to the two original crosses 1-3-12-14×12 and 3-2-4-1×13. Under each cross are grouped together the  $F_3$  progenies descended from each grandparent, the grandparents having been  $F_1$  plants 20 and 28 of the cross 1-3-12-14×12 and  $F_1$  plants 21 and 24 of the cross 3-2-4-1×13.

## THE THIRD GENERATION OF THE CROSSES

In each of the four  $F_2$  progenies grown in 1922, flowers on a number of individuals were bagged to insure strict self-fertilization. The selfed individuals were selected for average grade of petal spot as follows: (a) Highest, (b), most nearly intermediate, (c) lowest. Twenty-four  $F_3$  progenies, two representing each condition of petal spot in each  $F_2$  progeny, were grown in 1923. Eight parental progenies were grown in the same plat, each of the original parents of the hybrids having been represented by two progenies.

The frequency distributions for the several parental and  $F_3$  progenies are given in Table VI, the unit having been the average of several flowers graded on each individual plant. The grading was begun on July 14 and ended on August 18.

It is clear from the frequency distributions in Table VI that in every case the behavior of the  $F_3$  progeny accorded with the position of its parent in the  $F_2$  frequency distribution. The eight  $F_2$  parents having average grades higher than 7.5 proved to be pure dominants, the eight  $F_2$  parents having average grades lower than 0.5 proved to be pure recessives, and the eight  $F_2$  parents having average grades between 5 and 6.5 proved to be heterozygous. The percentages of spotless plants and departures from the expected 25 per cent in the segregating  $F_3$  progenies are stated in Table VII.

TABLE VII.—Percentages of spotless individuals and departures from the expectation in the segregating  $F_3$  progenies grown in 1923

$F_3$ progeny.	Number of plants.	Percentage spotless. <sup>a</sup>	Departure from the expectation (25 per cent).
(1-3-12-14×12) 20-27.....	39	20.5	4.5±4.33
(1-3-12-14×12) 20-35.....	33	21.2	3.8±4.82
(1-3-12-14×12) 28-14.....	48	25.0	0±4.21
(1-3-12-14×12) 28-53.....	43	39.5	14.5±5.03
(3-2-4-1×13) 21-7.....	44	22.7	2.3±4.25
(3-2-4-1×13) 21-29.....	45	17.8	7.2±3.85
(3-2-4-1×13) 24-33.....	45	31.1	6.1±4.65
(3-2-4-1×13) 24-35.....	42	33.3	8.3±4.90
8 $F_3$ progenies as one array.....	339	26.5	1.5±1.61

<sup>a</sup> The probable error of the percentage is omitted, being the same as that of the departure from the expectation.

Reference to Table VII shows that some of the  $F_3$  progenies gave rather wide departures from the expected 25 per cent of spotless individuals. In no case, however, is the departure mathematically significant. If all of the segregating  $F_3$  progenies are considered as one array, the departure from 25 per cent spotless is smaller than its probable error. As was the case in the  $F_2$  progenies taken as one array (Table V), it is the spotless class which is slightly in excess of the expectation.

The spotted portion (grades 5.5 to 9) of the frequency distributions of the heterozygous  $F_3$ 's (Table VI) is bimodal in 7 of the 8 progenies, indicating that the heterozygotes are partly distinguishable from the dominants. Further evidence that such is the case is afforded by the fact that all of the 8  $F_2$  plants selected in 1922 as nearest intermediate (grade 5.3 to 6.2) gave  $F_3$  progenies which segregated. While, for reasons stated in discussing the similar condition in  $F_2$ , the heterozygotes giving relatively high grades can not be distinguished with certainty from the dominants giving relatively low grades, it may be worth while to note that in the spotted portion of the whole  $F_3$  population there were 163 plants having an average grade not higher than 7 and 86 plants having an average grade higher than 7, a ratio of 1.9:1. While this classification is admittedly arbitrary, it indicates that the phenotypic ratio is 1:2:1.<sup>6</sup>

The statistical constants, as computed from the frequency distributions, of the parental and the homozygous  $F_3$  progenies are stated in Table VIII.

Comparing the means for groups of progenies having a common origin taken as one array (heavy-faced figures in Table VIII), it will be noted that the populations derived from the two dominant parents (12 and 13) of the original hybrids do not differ significantly, and that this is likewise true with respect to the populations representing the two recessive parents (1-3-12-14 and 3-2-4-1). Data given in Table IV show that in the preceding generation also the populations representing, respectively, the dominant and the recessive parents of the hybrids did not differ significantly in their means for petal spot. On the other hand, the population embracing the four dominant  $F_3$  progenies derived from the cross 1-3-12-14  $\times$  12 differs slightly, but significantly, in its means from that of the combined population of dominant  $F_3$ 's derived from the cross 3-2-4-1  $\times$  13, the difference having been  $0.30 \pm 0.028$ . Similarly, the mean for the combined recessive  $F_3$  progenies derived from the cross 1-3-12-14  $\times$  12 shows a small but probably significant difference from that of the combined recessive  $F_3$  progenies derived from the cross 3-2-4-1  $\times$  13, the difference having been  $0.21 \pm 0.055$ . In both cases it is the descendants of the cross 3-2-4-1  $\times$  13 which gave the high mean.

There are also significant differences among the several  $F_3$  progenies derived from the same original cross and even between progenies which had had the same  $F_1$  grandparent. Of the two dominant  $F_3$  progenies descended from (1-3-12-14  $\times$  12)  $F_1$  plant No. 20, progeny 20-14 gave a significantly lower mean than progeny 20-15 (difference  $0.4 \pm 0.067$ ). Of the two dominant  $F_3$  progenies descended from (3-2-4-1  $\times$  13)  $F_1$  plant No. 24, progeny 24-18 gave a significantly lower mean than progeny 24-46 (difference  $0.3 \pm 0.050$ ). Of the two recessive  $F_3$  progenies descended from (1-3-12-14  $\times$  12)  $F_1$  plant No. 28, progeny 28-32 gave a significantly higher mean than progeny 28-49 (difference  $0.5 \pm 0.086$ ). Of the two recessive  $F_3$  progenies descended from (3-2-4-1  $\times$  13)  $F_1$  plant No. 21, progeny 21-16 gave a significantly higher mean than progeny 21-22 (difference  $1.2 \pm 0.072$ ). Thus significant differences are found among the dominant or recessive descendants of each of the four  $F_1$  individuals which gave rise to the  $F_2$ 's grown in 1922 and the  $F_3$ 's grown in 1923.

<sup>6</sup> On this basis of classification in the spotted group, the whole segregating  $F_3$  population (8 progenies as one array) comprised 86 dominants, 163 heterozygotes, and 90 recessives.

TABLE VIII.—Statistical constants for petal spot grade of the parental and the homozygous  $F_3$  progenies grown in 1923

Population.	Number of plants.	Mean.	Standard deviation.
<b>DOMINANT PARENTAL</b>			
12-13-23.....	16	8.0 $\pm$ .05	.28
12-13-2.....	29	8.1 $\pm$ .04	.32
Combined.....	45	8.08 $\pm$ .032	.316
13-8-4.....	27	8.2 $\pm$ .04	.34
13-8-17.....	39	8.1 $\pm$ .04	.33
Combined.....	66	8.12 $\pm$ .028	.338
<b>DOMINANT <math>F_3</math></b>			
(1-3-12-14 $\times$ 12) 20-14.....	25	7.5 $\pm$ .06	.41
(1-3-12-14 $\times$ 12) 20-15.....	48	7.9 $\pm$ .03	.29
(1-3-12-14 $\times$ 12) 28-7.....	34	7.8 $\pm$ .04	.38
(1-3-12-14 $\times$ 12) 28-8.....	46	7.9 $\pm$ .04	.36
Combined.....	153	7.84 $\pm$ .021	.385
(3-2-4-1 $\times$ 13) 21-2.....	42	8.0 $\pm$ .04	.41
(3-2-4-1 $\times$ 13) 21-21.....	46	8.1 $\pm$ .03	.28
(3-2-4-1 $\times$ 13) 24-18.....	43	8.1 $\pm$ .04	.38
(3-2-4-1 $\times$ 13) 24-46.....	41	8.4 $\pm$ .03	.29
Combined.....	172	8.14 $\pm$ .019	.375
<b>RECESSIVE PARENTAL</b>			
1-3-12-14-2-23.....	28	1.9 $\pm$ .07	.57
1-3-12-14-2-25.....	26	2.3 $\pm$ .09	.67
Combined.....	54	2.10 $\pm$ .059	.646
3-2-4-1-22-1.....	26	2.1 $\pm$ .08	.63
3-2-4-1-22-27.....	25	1.8 $\pm$ .07	.49
Combined.....	51	1.97 $\pm$ .056	.590
<b>RECESSIVE <math>F_3</math></b>			
(1-3-12-14 $\times$ 12) 20-19.....	42	1.0 $\pm$ .05	.52
(1-3-12-14 $\times$ 12) 20-24.....	37	1.1 $\pm$ .05	.46
(1-3-12-14 $\times$ 12) 28-32.....	48	2.0 $\pm$ .07	.68
(1-3-12-14 $\times$ 12) 28-49.....	47	1.5 $\pm$ .05	.50
Combined.....	174	1.45 $\pm$ .035	.690
(3-2-4-1 $\times$ 13) 21-16.....	36	2.1 $\pm$ .06	.55
(3-2-4-1 $\times$ 13) 21-22.....	47	0.9 $\pm$ .04	.41
(3-2-4-1 $\times$ 13) 24-25.....	34	2.0 $\pm$ .07	.62
(3-2-4-1 $\times$ 13) 24-48.....	29	2.0 $\pm$ .08	.67
Combined.....	146	1.66 $\pm$ .042	.747

## SEGREGATION IN THE FIRST GENERATION

The data at hand indicate that there may have been a slight degree of segregation in  $F_1$ , although the evidence is not very conclusive, progenies of only two plants in each of the  $F_1$  progenies having been grown. Table IX gives, for each cross, the grades of the  $F_1$  parent individuals, the means of the spotted and spotless classes in their  $F_2$  progenies and the means of the dominant and recessive  $F_3$  populations derived from each  $F_1$  individual. Each pair of plants selected in  $F_1$  had differed by more than a half-grade. The differences between the corresponding  $F_2$  progenies are in the same direction but are smaller than in  $F_1$  and are not significant or barely significant. The differences between the  $F_3$  populations are not only in the same direction as in  $F_1$  and  $F_2$  but seem to be significant in all but one case.

TABLE IX.—Segregation in  $F_1$  as indicated by comparison of the means of the spotted and the spotless plants in the  $F_2$  progenies and of the means of the dominant and recessive  $F_3$  populations descended from each  $F_1$  plant

F <sub>1</sub> progeny.	F <sub>1</sub> parent and F <sub>2</sub> progeny No.	Grade of the F <sub>1</sub> parent.	Means of the 2 classes in F <sub>2</sub> .		Means of the F <sub>3</sub> populations. <sup>b</sup>	
			Spotted plants. <sup>a</sup>	Spotless plants.	Dominant.	Recessive.
1-3-12-14 × 12.....	20		7.06.71 ± 0.07	6.44 ± 0.05	7.79 ± 0.03	1.06 ± 0.03
1-3-12-14 × 14.....	28		7.66.96 ± 0.07	1.70 ± 0.06	7.89 ± 0.02	1.78 ± 0.04
Differences.....			.60.25 ± .10	.46 ± .08	.10 ± .04	.72 ± .05
3-2-4-1 × 13.....	21		7.26.90 ± 0.10	1.64 ± .13	8.06 ± 0.02	1.42 ± 0.03
3-2-4-1 × 13.....	24		7.97.11 ± 0.09	1.68 ± .07	8.23 ± 0.02	1.98 ± 0.03
Differences.....			.71.21 ± .13	.04 ± .15	.17 ± .03	.56 ± .07

<sup>a</sup> Dominant and heterozygous plants. <sup>b</sup> Each F<sub>3</sub> population comprises two progenies, taken as one array

## EVIDENCE OF MODIFYING FACTORS

The questions suggest themselves whether, in addition to the major factor determining full development of the spot as contrasted with its almost complete absence, there are minor factors which modify the degree of its expression; and whether such factors have been variously recombined in the hybrids.

No evidence of modifying factors is afforded by the results of selection during successive generations in the parental populations. In 1920, 1921, and 1922 the plant which gave the lowest average grade for petal spot in each of the recessive parental progenies was selfed and became the progenitor of a progeny grown the following year. Comparison of the parental values and the progeny means, as given in Table X, does not show a tendency to reduction of the spot. It will be shown presently that the apparent marked increase in the progenies grown in 1923 is probably attributable to the lateness of flowering of most of the plants in the parental progenies of that year.

In 1922 two plants were selfed in each of the parental progenies, these plants having represented the extremes of development of the spot for the population in question. Progenies of each of these plants were grown in 1923. The progeny means, as stated in Table XI, showed no differences of probable significance except in the third pair and in that case the higher mean was yielded by the progeny of the plant which had given the lower value.

TABLE X.—Grade of petal spot of selections in the recessive parental populations and mean grade of the progenies of these selections

Selection and year.	Grade of the selected individual.	Mean grade of its progeny grown the year following.
1-3-12, No. 14 (1920).....	0	1.2 ± 0.11
1-3-12-14, No. 2 (1921).....	0.7	.7 ± .04
1-3-12-14-2, No. 25 (1922).....	.4	2.3 ± .09
3-2-4, No. 1 (1920).....	0	.7 ± .06
3-2-4-1, No. 22 (1921).....	.2	.7 ± .03
3-2-4-1-22, No. 27 (1922).....	.2	1.8 ± .07

TABLE XI.—*Petal spot grade of pairs of plants selected in each parental progeny of 1922 as representing the extremes of the progeny and means of the progenies of these plants grown in 1923*

Progeny and selection.	Petal spot grade of the individual selected in 1922.	Mean of its progeny grown in 1923.
SPOTTED PARENTAL FAMILIES		
12-13 { No. 2 .....	7.3	8.1 ± 0.04
{ No. 23 .....	7.9	8.0 ± 0.05
Difference .....	.6	.1 ± .064
13-8 { No. 17 .....	7.2	8.1 ± .04
{ No. 4 .....	8.0	8.2 ± .04
Difference .....	.8	.1 ± .057
SPOTLESS PARENTAL FAMILIES		
1-3-12-14-2 { No. 25 .....	.4	2.3 ± .09
{ No. 23 .....	1.2	1.9 ± .07
Difference .....	.8	.4 ± .114
3-2-4-1-22 { No. 27 .....	.2	1.8 ± .07
{ No. 1 .....	.9	2.1 ± .08
Difference .....	.7	.3 ± .106

There are, however, indications of a recombination of modifying factors in the hybrids. Evidence has been presented that a slight degree of segregation took place in  $F_1$  and it has been mentioned (p. 499) that in four pairs of  $F_3$  progenies the means differed significantly, although each pair had the same  $F_1$  grandparent. Nine of the 16 homozygous  $F_3$  progenies, when compared with the population representing the respective dominant or recessive great grandparent (Table VIII) showed increase or diminution of the spot, the  $F_3$  mean having differed from the parental mean by an amount equal to four or more times the probable error of the difference. The most pronounced of these differences are stated in Table XII, there being two cases in which dominant and three cases in which recessive  $F_3$  progenies differed very significantly from the corresponding parental population. In one of the dominant  $F_3$ 's the spot was increased and in the other it was diminished. All three of the recessive  $F_3$ 's show a marked diminution of the spot, amounting in each case to a full grade.

This evidence from  $F_3$  can not be accepted without reservation, however, for the reasons that soil heterogeneity of the plat used in 1923 resulted in considerable differences in the rate of development and earliness of flowering of the plants in the various progenies and that the degree of expression of the spot appears to have been affected by the stage of development of the plant.

The mean grade of petal spot was determined separately for the retarded and for the earlier-flowering plants in 11 recessive parental and  $F_3$  progenies of 1923. In every progeny the more backward plants gave a higher mean grade than the more advanced plants, the average differ-

ence for all 11 progenies having amounted to 0.5 grade and the difference having been significant in 8 of the progenies. A similar comparison in 10 dominant parental and  $F_3$  progenies showed no consistent tendency to greater development of the petal spot in the retarded as compared with the earlier-flowering plants.

TABLE XII.—Evidence of increase or diminution of the petal spot in nonsegregating  $F_3$  progenies as compared with populations representing the respective dominant or recessive parent of the cross

Population. <sup>a</sup>	Mean grade of petal spot.	Difference between means, showing petal spot in F <sub>3</sub> as compared with parental population to have been—	
		Increased.	Diminished.
DOMINANTS			
Parental 13-8.....	8.1 ± 0.038	0.3 ± 0.042	.....
F <sub>3</sub> 24-46.....	8.4 ± 0.031		
Parental 12-13.....	8.1 ± 0.032	.....	0.6 ± 0.064
F <sub>3</sub> 20-14.....	7.5 ± 0.055		
RECESSIVES			
Parental 1-3-12-14-2.....	2.1 ± 0.059	.....	1.1 ± 0.080
F <sub>3</sub> 20-19.....	1.0 ± 0.054		
Parental 1-3-12-14-2.....	2.1 ± 0.059	.....	1.0 ± 0.078
F <sub>3</sub> 20-24.....	1.1 ± 0.051		
Parental 3-2-4-1-22.....	2.0 ± 0.056	.....	1.1 ± 0.069
F <sub>3</sub> 21-22.....	.9 ± 0.040		

<sup>a</sup> The parental population in each case comprises two progenies taken as one array.

As a measure of the comparative earliness of the several populations, the mean number of flowers per plant during the first four days of the grading period (July 14-17) was computed for each parental population and homozygous  $F_3$  progeny. The correlation between the progeny means for number of early flowers and grade of petal spot was then computed separately for the 10 recessive and for the 10 dominant parental and  $F_3$  populations. There was found to be a pronounced negative correlation in the case of the recessives, the coefficient having been  $-.67 \pm .10$ . In other words, recessive progenies in which many of the plants were late in development tended to have a relatively high mean for petal spot. The dominant populations, on the other hand, showed an entire absence of correlation between earliness and grade of petal spot.

Both the dominant and the recessive parental populations, which were situated together at one end of the plat, proved to be inferior to most of the  $F_3$  progenies in flower production at the beginning of the grading period. Many of the plants in the parental progenies did not begin to flower, or flowered very sparingly, until near the end of the grading period. Since, in the spotless populations, retardation of growth tends to a more pronounced expression of the spot, it is obvious that the means for petal spot grade of backward and of more advanced recessive popu-

lations are not fairly comparable. This objection applies to all comparisons of recessive  $F_3$  progenies with recessive parental populations in Table XII, the former having had, in every case, a higher rate of early flowering than the latter. This factor of relative earliness of the plant also affects the comparisons between recessive  $F_3$  progenies which had the same  $F_1$  grandparent. Of the two pairs of recessive  $F_3$ 's mentioned on page 499 as showing significant differences in mean grade of petal spot (28-32 and 28-49, 21-16 and 21-22), in each case the progeny which gave the lower mean for petal spot had the higher rate of early flowering.

On the other hand, the evidence of the occurrence of modifying factors from comparisons involving dominant  $F_3$  progenies is not vitiated by differences in earliness, since in the dominant populations a retarded condition of the plants apparently did not result in a more pronounced expression of the petal spot. Of the two comparisons of dominants in Table XII, one ( $F_3$  24-46) gave a significantly higher and the other ( $F_3$  20-14) gave a significantly lower mean grade of petal spot than the corresponding dominant parental population, yet the rate of early flowering in both  $F_3$ 's had resembled the parental rate. Comparison with one another of the dominant  $F_3$  progenies 20-14 and 20-15, both of which had the same  $F_1$  grandparent, shows that the latter gave a significantly higher mean for petal spot grade, although it had much the heavier rate of early flowering. The relations of earliness and development of the petal spot are in this case the reverse of what was noted in the recessive populations.

Returning to the indications of segregation in the first generation afforded by a correspondence between the differences in  $F_1$  and  $F_3$  (Table IX) it may be said that, in this case, the factor of relative earliness did not operate consistently in the recessive populations. In the cross 1-3-12-14  $\times$  12 the recessive  $F_3$  descendants of  $F_1$  plant 28 gave a very significantly higher mean for petal spot than the recessive  $F_3$  descendants of  $F_1$  plant 20; and in the cross 3-2-4-1  $\times$  13 the recessive  $F_3$  descendants of  $F_1$  plant 24 gave a very significantly higher mean for petal spot than the recessive  $F_3$  descendants of  $F_1$  plant 21. But the heavier rate of early flowering was shown in the first cross by the population which gave the higher mean for petal spot and in the second cross by the population which gave the lower mean for petal spot.

The position as regards modifying factors for petal spot in this material may be summed up in the statement that there are indications, but not conclusive proof, of the existence of such factors which, by their segregation and recombination, have brought about a slight degree of differentiation in the hybrids.

#### VARIATION IN PETAL SPOT ON THE INDIVIDUAL PLANT

The frequency distributions based upon averages of from 3 to 10 flowers per plant (Table VI) show a gap, amounting to 2.5 grades, between the plants which gave the lowest averages in the dominant parental and  $F_3$  populations and the plants which gave the highest averages in the corresponding recessive populations. The gap is bridged, however, if individual flowers be considered. This is shown by the data presented in Table XIII, which gives the grades of the individual flowers grading lowest in the 12 dominant and highest in the 12 recessive parental and  $F_3$  progenies of 1923.



TABLE XIII.—*Overlapping of the dominant and recessive populations of 1923 in grade of petal spot of the individual flowers*

Group.	Total number of flowers graded.	Numbers of flowers grading.		
		4.0	4.5	5.0
Dominants.....	3,843	9	3	15
Recessives.....	3,700	97	5	1

The average degree of variation on the individual plant may be shown by computing, for a given population, the mean of the differences between the flower graded highest and the flower graded lowest on each individual. In computing these means, plants were disregarded on which fewer than ten flowers were graded. The means were computed separately for the dominant and for the recessive parental and  $F_3$  populations, each as one array, and for the dominant, heterozygous and recessive plants in the segregating  $F_3$  progenies,<sup>7</sup> giving, in all, 5 populations of which the mean and maximum variation, on the individual plant, are stated in Table XIV.

TABLE XIV.—*Mean and maximum variation of petal spot grade on the individual plants in the dominant and recessive parental and  $F_3$  populations and in the dominant, heterozygous and recessive classes of the segregating  $F_3$  progenies.*

Population.	Number of plants.	Range of grades on the individual.	
		Mean.	Maximum and (in parenthesis) the number of plants showing it.
Dominant parental and $F_3$ .....	297	$2.10 \pm 0.035$	5.0 (6)
Recessive parental and $F_3$ .....	278	$2.64 \pm .020$	5.0 (1)
Dominants in segregating $F_3$ 's.....	62	$2.18 \pm .060$	4.5 (2)
Heterozygotes in segregating $F_3$ 's.....	107	$2.55 \pm .057$	5.0 (3)
Recessives in segregating $F_3$ 's.....	66	$2.42 \pm .057$	4.0 (2)

It is noteworthy that variation amounting to four or five grades is shown by a few individuals in each population. Comparison of the mean ranges shows significantly less variation in the dominant than in the recessive population. The dominants in the segregating  $F_3$  progenies also are individually less variable than the heterozygotes and the recessives, although the difference between dominants and recessives is, in this case, slightly less than three times its probable error. The smaller variation of the dominants may be apparent rather than real, for the reason that slight differences are less easily detected when the spot is strongly developed than when it is weakly developed.

<sup>7</sup> Plants giving an average grade of 7.3 or higher were taken as dominant, 7.3 having been the lowest average given by any plant in the dominant parental populations. Plants giving an average grade lower than 7 and higher than 5 were taken as heterozygotes, the comparatively few individuals which averaged 7 to 7.2 having been left out of account as of uncertain classification. It is believed that classification on this basis is sufficiently accurate for the purpose in view. There could be no question as to the identity of the recessives.

The variation in grade of petal spot on the individual plant appears not to be due solely to variation in factors of the external environment. This is indicated by comparing flowers graded on the same plant on the same day. Of such comparisons, in 1923, 75 showed a difference of 2 grades, 31 of  $2\frac{1}{2}$  grades, 24 of 3 grades, 6 of  $3\frac{1}{2}$  grades, 2 of 4 grades and 3 of 5 grades. It may be argued, however, that since it is a rare occurrence for more than one flower to open on the same fruiting branch on the same day, these differences in the petal spot of flowers opening simultaneously on the same plant may be attributed to variations in the environment, either external or internal, during the period when the successive fruiting branches were being developed.

The  $F_1$  hybrid progenies grown in 1921 and the  $F_2$  hybrid progenies grown in 1922 did not show greater mean variation in petal spot on the individual plant than did the corresponding parental progenies. These facts, together with the absence of significantly greater individual variation in the heterozygous than in the recessive plants of the segregating  $F_2$  progenies grown in 1923 (Table XIV) indicate that the type of "vegetative segregation" recently described by Gates<sup>8,9</sup> does not occur in this case.

#### RELATION OF PETAL SPOT TO SIZE OF THE FLOWER

It has been noted, in normal Pima populations, that the petal spot often is less well developed in the small and sometimes misshapen flowers borne by stunted plants than in the larger flowers borne by well-grown plants. The spotless families described in this paper showed no apparent inferiority to the spotted families in the vegetative vigor and fruitfulness of the plants, when grown under comparable conditions. It has been observed, however, in grading petal spot in the spotless populations that flowers showing no trace of the spot often have a small corolla and relatively few and small stamens, while flowers of normal size in the same populations often exhibit a faintly developed spot.

Comparison of the mean corolla length of spotted plants and of spotless plants in the  $F_2$  progenies of 1922 showed a difference of only  $1.5 \pm 0.39$  mm. in favor of the former, the mean length having been  $60.4 \pm 0.20$  mm. in the spotted class (276 flowers) and  $58.9 \pm 0.33$  mm. in the spotless class (123 flowers). In 1923 a dominant and a recessive  $F_2$  progeny were compared, with negative results. The two progenies, descendants of the same  $F_1$  plant, were situated in adjacent and continuous rows, hence soil heterogeneity was not a factor. One corolla was measured on one plant in each progeny, 41 flowers having been measured in the dominant progeny and 42 flowers in the recessive progeny. The mean corolla length was  $62.3 \pm 0.36$  in the former and  $63.0 \pm 0.39$  in the latter, the difference amounting to only 1.3 times its probable error.

Although these comparisons of the mean corolla length of the spotted and of the spotless populations showed little or no difference, a significant positive correlation between grade of petal spot and length of corolla was found to exist within each class. In the  $F_2$  progenies of 1922, 276 flowers of the spotted class on which both characters were measured

<sup>8</sup> GATES, R. Ruggles. VEGETATIVE SEGREGATION IN A HYBRID RACE. *In* Jour. Genetics, v. 6, p. 237-253, pl. 9. 1917. List of references, p. 252-253.

<sup>9</sup> A PECULIAR TYPE OF VARIABILITY IN PLANTS. *In* Jour. Genetics, v. 13, p. 13-45, 24 fig. 1923. Literature cited, p. 44-45.

yielded a coefficient of correlation of  $0.645 \pm .024$  and 123 flowers of the spotless class gave a coefficient of  $0.307 \pm .055$ .<sup>10</sup>

#### ENVIRONMENTAL REACTIONS OF THE PETAL SPOT

There is good reason to believe that both the size and the intensity of color of the petal spot vary in response to variations in the physical environment, although this has not been tested by controlled experiments. Soil factors play a part, not only indirectly, by affecting the rate of development of the plant (p. 502) but probably in a more direct manner. Thus it has been observed that in Pima cotton fields the petal spot is less well developed where the plants are stunted than where they have made a normal growth, although the same stock of seed was used in planting the entire field. It has been shown that a positive correlation exists between size of flower and development of the spot, and since the flowers borne by stunted plants usually are small, the comparatively weak development of the spot on such plants may be regarded as part of a general reduction caused by the unfavorable environment.

The influence of meteorological factors will be examined, first, by comparing the means for petal spot in different years and, second, by comparing the earlier and the later flowers produced by the same individual plant during the same season.

Data given in Table I indicate that conditions in some years favor a more pronounced expression of the petal spot, the mean grade in both spotted and spotless populations, but especially the latter, having been notably higher in 1923 than in other years. It was pointed out, in discussing the evidence for the occurrence of modifying factors, that the lateness of flowering of the plants in the spotless parental progenies of 1923 may have been a factor contributing to the relatively high mean of that year. Yet the more pronounced development of the spot in 1923 can not be attributed wholly to this factor, for the spotted parental population, in which the backward plants did not seem to have the spot increased as was the case in the spotless population, also gave a significantly higher mean than in previous years. Comparison of the spotless populations of successive years in respect to the percentages of flowers showing no trace of the spot gives further evidence that conditions in 1923 favored an exceptionally high degree of expression of the spot. Because of the retarded growth of most of the plants in the parental populations of 1923, the recessive  $F_3$  progenies, in which most of the plants had shown a normal rate of growth, were compared with the parental populations of the two preceding years. The percentages of entirely spotless flowers were as follows, the figures in parenthesis indicating the total numbers of flowers graded: 1921 (290) 43.8 per cent; 1922 (561), 45.6 per cent; 1923 (2,826), 16.1 per cent.

In endeavoring to ascertain whether the degree of expression of the petal spot differs in different periods of the same season, comparison was made in 1922 of the means based upon the first 5 flowers and the second 5 flowers graded on the same individual plants. The two means were computed separately for 143 dominant plants and for 85 recessive plants.

<sup>10</sup> Corolla length and petal spot were correlated positively and significantly in a hybrid between upland and Egyptian cottons, the coefficient of correlation for 180  $F_2$  plants having been  $0.244 \pm .047$ . The correlations of petal spot with 37 other characters were determined on this material and in all but two cases the coefficient of correlation was less than 3.5 times its probable error. The correlations in question were with corolla length and with calyx dentation,  $r$  in the latter case having been  $-.220 \pm .048$ . KEARNEY, Thomas H. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. Dept. Agr. Bul. 1164, p. 45, Table 12. 1923.

The means for the dominant plants showed no difference between the earlier and the later flowers but, for the recessive plants, the mean for the earlier flowers was  $0.24 \pm .039$  grade higher than the mean for the later flowers. A similar comparison in 1923 on 285 dominant and 267 recessive plants on which 10 flowers per plant had been graded showed, in both groups, a difference of 0.1 grade in favor of the earlier flowers, the difference in one case having been 6 and in the other case  $4\frac{1}{2}$  times its probable error. In both years, therefore, comparison of the earlier with the later flowers on the same plants gave evidence of a mathematically significant but practically unimportant difference in grade of petal spot in favor of the earlier flowers.

The fact that the early, as compared with the later flowers produced by the same individual, tend to give a slightly higher average grade of petal spot seems at first glance inconsistent with the fact previously noted (p. 502) that the petal spot is apt to be better developed on retarded plants than on plants which have grown more rapidly. The apparent contradiction disappears if we consider that the earlier flowers on the more advanced plants were produced when these plants were in a stage of growth more nearly comparable to that of the retarded plants.

A more marked reduction in the petal spot takes place towards the end of the flowering season. On October 6, 1923, it was noted that in both the dominant and recessive populations the flowers showed a pronounced diminution in the size and intensity of the spot as compared with the condition on August 18, when the grading ended.

#### FREQUENCY OF 4-LOCK BOLLS IN RELATION TO PETAL SPOT

Observation of the growing plants and measurement of the leaves, bolls and fiber have shown that the families which furnished the spotless parents of the hybrids here described are typical Pima except in the development of the petal spot and the proportion of 4-lock bolls. In other color characters of the flowers, greenish yellow color of the petals and empire yellow color of the pollen, they show no departure from the type. In percentage of 4-lock bolls, the descendants of both of the spotless selections of 1917 exceed the average for the variety as grown at Sacaton. This was shown by data from the progenies grown in 1919 and 1920, in comparison with the general stock of Pima cotton.<sup>11</sup> Additional evidence was obtained in 1921 and 1922, by comparing the spotless parental progenies with progenies representing the normal or spotted parents of the hybrids. Data for both the parental and the hybrid populations are given in Table XV.

It is clear from the data in Table XV that in 1921 and 1922 the progenies representing the two spotless families had significantly higher mean percentages of 4-lock bolls than the progenies descended from the spotted parents of the crosses, just as, in 1919 and 1920, the spotless families had significantly higher percentages than the "bulk" Pima with which they were compared. It is also evident that the descendants of spotless plant No. 3 continue to give very significantly higher means for this character than the descendants of spotless plant No. 1. In  $F_1$ , the percentage of 4-lock bolls in one of the crosses resembles that of the progeny representing its spotless parent, while the other cross gave an almost intermediate percentage. In  $F_2$ , the mean percentage of 4-lock

<sup>11</sup> KEARNEY, Thomas H. HERITABLE VARIATIONS IN AN APPARENTLY UNIFORM VARIETY OF COTTON. In *Jour. Agr. Research*, v. 21, p. 237-241. 1921.

bolts in both crosses is somewhat below the average of the parental means, but is significantly higher than the percentage of the normal or spotted parent. The heritability of this character is shown further by the fact that in  $F_2$  the cross involving 3-2-4, the spotless family having the higher percentage of 4-lock bolts, gave a mean percentage of 4-lock bolts nearly twice as great as the cross involving spotless family 1-3-12. Expressed as a percentage of the lower mean, the difference between the hybrids ( $F_2$ ) amounted to 89 per cent while the difference between the spotless parental progenies grown the same year (1922) amounted to 131 per cent.

TABLE XV.—Percentages of 4-lock bolts in the parental and hybrid populations grown in 1921 and 1922

Populations of 1921.	Number of plants.	Mean percentage, 4-lock bolts.	Populations of 1922.	Number of plants.	Mean percentage, 4-lock bolts.
PARENTAL			PARENTAL		
1-3-12-14 (spotless)...	14	4.15 ± 0.34	1-3-12-14-2 (spotless)	22	4.54 ± 0.37
12 (spotted).....	20	2.45 ± .21	12-13 (spotted).....	19	1.84 ± .15
Difference.....		1.70 ± .40	Difference.....		2.70 ± .40
HYBRID ( $F_1$ )			HYBRID ( $F_2$ )		
1-3-12-14 × 12.....	28	4.50 ± .28	1-3-12-14 × 12 <sup>a</sup> .....	83	2.71 ± .13
PARENTAL			PARENTAL		
3-2-4-1 (spotless).....	27	9.31 ± .33	3-2-4-1-22 (spotless).....	26	10.50 ± .53
13 (spotted).....	14	2.92 ± .24	13-8 (spotted).....	22	2.44 ± .24
Difference.....		6.39 ± .41	Difference.....		8.06 ± .58
HYBRID ( $F_1$ )			HYBRID ( $F_2$ )		
3-2-4-1 × 13.....	33	5.87 ± .30	3-2-4-1 × 13 <sup>a</sup> .....	62	5.13 ± .23

<sup>a</sup> Two progenies as one array.

There appears to be no general correlation, either negative or positive, between the degree of development of the petal spot and the mean number of boll locks per plant. In  $F_1$  and  $F_2$  of a hybrid between upland and Egyptian cottons, the coefficients of correlation for this pair of characters were, respectively,  $-0.043 \pm 0.137$  and  $0.011 \pm 0.050$ . As concerns the material dealt with in this paper, the coefficient of correlation for 125 plants in the progenies of the spotless families grown in 1920 was  $0.069 \pm 0.060$ . The correlations were also worked out for the spotted (dominant and heterozygous) plants in the  $F_2$  progenies of 1922, taking as one array in each case the two progenies of the cross 1-3-12-14 × 12 and the two progenies of the cross 3-2-4-1 × 13. The numbers of plants were, respectively, 55 and 48 and the coefficients of correlation obtained were  $-0.045 \pm 0.091$  and  $0.148 \pm 0.095$ .<sup>12</sup> A further indication of the absence of linkage was afforded by comparison of the mean percentages of 4-lock bolts of the spotted and the spotless segregates in each  $F_2$  progeny. There was in no case a significant difference between the two

<sup>12</sup> The correlations worked out in the upland × Egyptian hybrid were between petal spot grade and mean number of boll locks per plant, while in the crosses described in this paper the correlations were between petal spot grade and percentage of 4-lock bolts. These are merely different expressions of the same relation, practically all of the bolts in this variety of cotton having either three or four locks.

means. Finally, the percentage of 4-lock bolls was determined in 1923 for the homozygous  $F_3$  progenies 21-21 and 21-22, the former dominant and the latter recessive for petal spot. Soil heterogeneity was not a factor in this comparison, the two progenies having been grown in adjacent and conterminous rows. The percentages were  $5.11 \pm 0.33$  and  $5.33 \pm 0.34$ , the difference having been much smaller than its probable error.

The two plants of 1917 which became the progenitors of the two spotless families were selected late in the fall in the course of a search for plants having exceptionally numerous 4-lock bolls. The unusual character of their flowers was first noticed in their progenies. The spotless character is extremely rare in the Pima variety and, indeed, has not been observed except in the descendants of these individuals. Considering this fact and the absence of linkage between spotless petal and a high percentage of 4-lock bolls, the coincidence would be an extraordinary one if the original spotless plants had been unrelated. It is much more probable that, although discovered almost by accident in a field grown from "bulk" seed, they were both descended from an individual presenting a chance combination of the two characters.

#### DISCUSSION

Comparatively few characters of the cotton plant have been proved conclusively to segregate in definite Mendelian fashion. It has seemed worth while, therefore, to present rather fully the evidence that the character petal spot affords an example of such behavior. The sharpness of the segregation in this case is interesting because the recessive allelomorph usually is expressed in a faintly spotted rather than an absolutely spotless condition. It is also of interest that the character proves to be decidedly variable in the homozygous populations, whether recessive or dominant, variation being shown not only from plant to plant and in response to environic influences, but among flowers produced simultaneously on the same individual plant.

Petal spot, unlike such characters as fruitfulness, size of the boll and length and abundance of the fiber, is of no direct agricultural or commercial importance. Nevertheless, any character which may serve as the "hall mark" of an agriculturally desirable strain or variety is likely to prove useful. This is particularly the case in a crop like cotton, since the commercial value of the product depends largely upon its uniformity and this, in turn, upon the genetic purity of the stock. Cotton is subject to cross-pollination and, consequently, in order to maintain pure planting seed of a variety, it is often necessary to rogue seed-increase fields which have been grown in proximity to another variety or stock, in order to eliminate accidental hybrids. It is obvious that roguing can be done thoroughly only if the stock to be rogued possesses characters which make it easy to distinguish the hybrids resulting from cross-pollination with other stocks. It is also important that the distinguishing characters be recognizable early in the summer, before there has been opportunity for extensive cross-pollination of the parent stock by the hybrids present in the same field.

The Pima variety is so uniform morphologically that it is usually impossible to detect, in time for roguing, the offspring of accidental crosses between strains which have been selected on the basis of fruitfulness, earliness and properties of the fiber and seeds. Such a character as spotless petal meets all the requirements of a serviceable "hall mark" for its

allelomorph, full-spotted, is almost completely dominant. Consequently the first-generation heterozygotes resulting from accidental cross-pollination with a normal stock of the variety could be recognized as soon as the first flowers open.

Since spotless petal is a simple recessive, there should be no great difficulty in transferring this character to any other strain of the Pima variety. Complications would ensue were spotless associated with marked inferiority in characters of practical importance. Fortunately, the spotless families described in this paper are satisfactory in fruitfulness and in the length, strength, and abundance of the fiber, hence there is no reason to expect that the offspring of such a cross would be inferior, either agriculturally or commercially.

#### SUMMARY

A conspicuous red spot near the base of the petal characterizes Sea Island and Egyptian cottons, while the petals of most varieties of upland cotton are spotless. In a hybrid between the Holdon variety of upland cotton and the Pima variety of Egyptian cotton, this character showed unifactorial segregation, the ratio of spotted to spotless plants in  $F_2$  having been approximately 3:1.

Two plants, discovered in 1917 in a field of Pima cotton, gave rise to families breeding true for a spotless or very faintly spotted petal. Crosses between these families and an inbred family in which the spot is fully developed gave opportunity for study of the inheritance of this character within the Pima variety.

In the first generation of the hybrid the spot was almost but not quite dominant, the hybrid means having been significantly lower than those of the spotted parental populations. The variability of the hybrid was of the same order of magnitude as that of the parental families.

The second generation of the crosses showed very definite segregation into a spotted and a spotless (strictly speaking a very faintly spotted) class, the percentages of spotless individuals not departing significantly from the 25 per cent expected with a single factor difference. The variability within the spotted class was, however, greater than in the populations representing the spotted grandparents, and in two of the four  $F_2$  progenies the frequency distributions of the spotted segregates were bimodal. These facts indicate that the heterozygotes are partly distinguishable from the pure dominants.

In the third generation 24 progenies were grown from plants in each  $F_2$  progeny representing, respectively, the most spotted, least spotted and most nearly intermediate condition. The behavior in  $F_3$  was in complete accord with the expectation. The eight progenies of fully spotted  $F_2$  plants bred true, as did the eight progenies of spotless  $F_2$  plants, while there was sharp segregation in each of the eight progenies of  $F_2$  plants which were most nearly intermediate. The percentages of spotless individuals in the segregating  $F_3$  progenies in no case departed significantly from the expected 25 per cent. When all of the segregating progenies are taken as one array the percentage of spotless individuals is  $26.5 \pm 1.6$ . The proof is conclusive that the spotless or very faintly spotted condition is a simple recessive.

The spotted class in seven of the eight segregating  $F_3$  progenies gave a bimodal distribution, this fact confirming the evidence from  $F_1$  and  $F_2$  that full-spotted is not completely dominant and that the heterozygotes are partly distinguishable.

The question whether modifying factors are involved, in addition to the major factor which determines presence of the spot as contrasted with its complete or nearly complete absence, was not answered decisively. There were indications of a slight degree of segregation in  $F_1$  and some of the dominant and recessive  $F_2$  progenies showed apparently significant increase or diminution of the spot in comparison with the corresponding parental population. But it was found that environmental factors had contributed to these differences in  $F_2$  by influencing the rate of development of the plants in the several populations. Furthermore, selection within the parental lines appears to have resulted in no perceptible modification of the spot. Yet, although the evidence at hand does not prove the existence of modifiers, it by no means justifies the conclusion that such factors are not involved.

The character petal spot shows considerable variation on the individual plant, even among flowers which open on the same day. The variation was not greater in the heterozygous than in the homozygous populations.

The degree of expression of the petal spot was found to be correlated positively and significantly with size of the flower, in both spotted and spotless populations. There was, however, no evidence of linkage.

The spot is affected by the environment, being responsive to soil variations and also to seasonal changes. Differences were noted both as between different seasons and as between different periods of the same season.

The spotless families are typical Pima in all other characters, except that they have given consistently a higher proportion of 4-lock bolls than normally spotted Pima populations grown under comparable conditions at the Sacaton Station.

There is an entire absence of linkage between spotless petal and a relatively high mean number of boll-locks and the spotless condition has been observed only in the two families described in this paper. It is probable, therefore, that the progenitors of these families, although discovered in a population grown from "bulk" seed, were nearly related if not sister plants.

Such a character as spotless petal should be useful as the "hall-mark" of an agriculturally and commercially valuable stock of Pima cotton. The fact that spotless is recessive would make it easy, in roguing seed-increase fields of a strain possessing this character, to recognize the first generation hybrids resulting from accidental cross-pollination with normal Pima, since they would have the spot well developed.

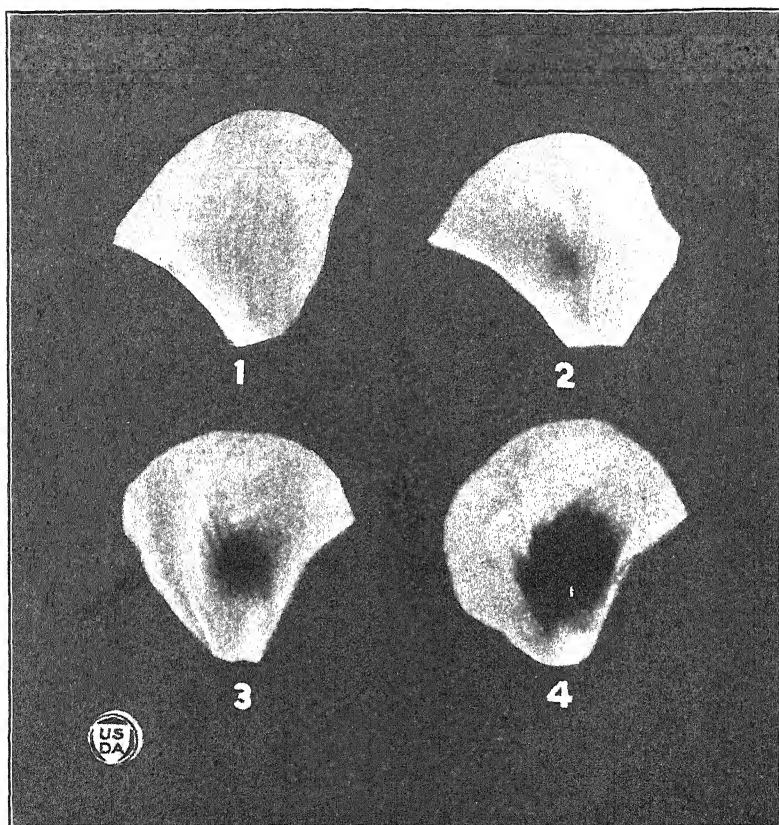
The recessive nature of spotless should make it readily transferable by hybridization to any desirable strain of this variety. No unfavorable results from such combinations need be anticipated, for the existing spotless families are not inferior to the average of the variety in fruitfulness and in the properties of the fiber.





# PLATE 1

Basal portions of petals of Pima cotton showing the range of grades in the spotless and normal families and in the hybrid between them. Grades 0 (fig. 1) and 3 (fig. 2) represent approximately the extremes of the recessive populations, while grades 6 (fig. 3) and 9 (fig. 4) represent approximately the extremes of the dominant populations.





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## BREEDING, FEEDING, AND OTHER LIFE HABITS OF MEADOW MICE (*MICROTUS*)<sup>1</sup>

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### INTRODUCTION

The meadow mice, field mice, or ground voles, of the genus *Microtus* (2),<sup>2</sup> comprising numerous species and geographic varieties, are widely distributed throughout Europe, Asia, and North America, mainly in Temperate and Boreal Zones. Comparatively few species live in the Tropical or Lower Austral Zones, but many range north far beyond the Arctic Circle. Their greatest abundance and importance, however, is in the middle zones, the region of greatest agricultural production.

These rodents are all small or of medium size, with a stout compact body, short legs, short tail and ears, small eyes, soft fur, and dull colors, as becomes ground dwellers and habitual burrowers into the earth. Some are semiaquatic, living in marshes or along the banks of streams; others occupy the meadows, uplands, and even the semiarid parts of our desert areas. With this wide range of adaptation one or two, or sometimes three or four, species in a locality occupy most of the fertile areas of the United States and Canada, where they become of economic importance as farm and orchard pests (4; 5, p. 121-123; 9, p. 97-102; 10; 12; 20).

### MOUSE PLAGUES

In the Old World, meadow mice have for centuries presented serious agricultural problems, suddenly appearing in vast hordes that devoured the crops over extensive areas and causing heavy losses and much human suffering. Full accounts of these mouse plagues, which have appeared in Scotland, England, France, Germany, Italy, Greece, Hungary, Russia, Siberia, and Kamchatka, have been published.<sup>3</sup>

In our own country there are constant waves of abundance and diminution in numbers of meadow mice, not country-wide, but rising and falling locally like swells of the ocean. These are noted in frequent complaints of unusual damage to crops or fruit trees by mice in different parts of the country, and some of the higher waves have been designated as mouse years or mouse plagues (16).

One of these waves occurred during 1907 in the Humboldt Valley, Nevada (16), a rich agricultural area then under irrigation and supporting enormous areas of alfalfa that would yield two or three heavy cuttings a year with an aftermath for pasture during a part of the mild

<sup>1</sup> Received for publication, February 8, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 534-535.

<sup>3</sup> For references to accounts of mouse plagues in Old World countries, see list of literature in Lantz's report on field mice (11, p. 64).

winters. The ranches were large, mainly from 500 to 1,000 acres each, and some with as many as 4,000 acres. Cultural methods were lax at the time, and ditch banks, fence rows, and roadsides were a tangle of dense vegetation that remained uncut throughout the year.

Under this wealth of cover and with the rich food of alfalfa, the native meadow mice (*Microtus montanus*) became abundant during the fall of 1906, but were little noticed. By the early summer of 1907 the fields were full of them and only half a crop was harvested in the June cutting, and in August still less, and over whole ranches none. In the valley below Lovelock, Nev., crops and orchards had been destroyed or seriously injured, and many of the fields were bare of vegetation in November, 1907, and the millions of mice were digging deep into the ground for the alfalfa roots. In some of the fields there were literally thousands of mice to the acre.

The money loss to farmers from mice in that part of the valley was conservatively estimated at a quarter of a million dollars. Mice were also reported as very numerous and destructive to crops in the Carson and Paradise Valleys and at other points farther up the Humboldt Valley, but these areas were not included in the estimates of losses. A similar outbreak of these mice was reported by the ranch owners in 1899 and 1900, with almost as heavy losses as in the one cited.

A poisoning campaign was instituted by the Biological Survey of the United States Department of Agriculture under the expert supervision of Stanley E. Piper. The farmers combined to distribute poisoned food until the numbers of mice were reduced to a point where the situation could be controlled by their natural enemies, the coyotes, foxes, bobcats, badgers, skunks, weasels, owls, hawks (7, 8), ravens, crows, magpies, gulls, herons, and shrikes, which had congregated in unusual numbers to feast on them. By the next spring the mice were scarcely more numerous than usual. Their food supply and protecting cover had been consumed during the winter and reproduction had diminished while their enemies multiplied.

#### STUDY OF HABITS

To understand more fully the underlying causes of such rapid increases in numbers of these rodents, which are referred to generally in the literature dealing with field mice (1, p. 341-346; 6, p. 292-299; 13, p. 174-177; 14, p. 403-406; 15, p. 275-276; 17, p. 186-191; 18, p. 97-101; 19, p. 515-537), the Biological Survey has undertaken studies of the breeding and feeding habits of the common eastern representative of the group, *Microtus pennsylvanicus*, which with its subspecies covers over half of the continent of North America. To recount these experiments in detail would be tiresome and needless, for the general results are sufficiently startling in significance and practical application.

#### GENERAL HABITS OF MICROTUS PENNSYLVANICUS

The Pennsylvania meadow mice are primarily meadow dwellers. They are capable, however, of adapting themselves to almost any situation where a choice food supply is obtainable, and live in flooded marshes where they are forced to swim from one dry spot to another, cross small streams, or even range out over dry uplands in fields or orchards. They are active throughout the year; in winter, with thick fur coats, enjoying the restricted life under the deep snows in the north as much as their greater freedom with its increased dangers in summer. They dig nu-

merous shallow burrows in the ground, connected by a network of tiny roadways under the old grass and fallen vegetation, which protects them from the view of overhead enemies; build soft clean nests of fine grass and plant fibers under or on the surface of the ground, and in these nests raise their large families of young, and generally live a sociable, communistic, primitive type of animal life. While not highly endowed with even rodent intelligence, they are quick to adapt themselves to the food supply that is available, to the best possible means of protection from enemies, and to make the best of what, from the point of view of even a squirrel, might seem a very humble existence.

#### VOICES

Meadow mice are by no means dumb, as some have asserted, though to our gross ears they may seem so. The young have many forms of minute whimpering, whining, crying sounds which seem to have a meaning to their mother. As she leaves the nest, and perhaps interrupts their meal, there is a fine little complaining jumble of whimpers from the very young, to which she pays no attention. If one tumbles out of the nest and lies wriggling helplessly on the floor it cries with a vigor that brings a quick parental response and is carried back and replaced in the nest. If it falls far enough to be slightly hurt but not to be greatly injured a sharp squeal of pain sets the mother frantic to find and help it.

The adults and the older young have little talky squeaks and sharp cross squeaks and savage squeals, and in a fight a blur of squeaks and squeals and guttural growls, not far different from a dog fight on a very small scale. Then there are chatterings of teeth at each other and stampings and scratchings on the ground when rivals meet, all of which, and probably much more that we miss, have an evident meaning to them. A stranger or friend is recognized, either by voice, odor, sight, or other token, as quickly as we recognize a friend or foe.

#### DISPOSITIONS

In their home life these mice are sociable, friendly, playful, and happy among the members of a well-fed family or colony, but are savage fighters in the case of rival males or of females in defense of young, or among strangers. When hungry or without comfortable nests or living conditions, they become cross and quarrelsome, will fight among themselves, even to the death, and will eat those killed, especially the young.

They are fond of meat at any time, have no scruples against cannibalism, and will generally kill and eat newly born young not protected by the mother. Being ready fighters and always on the defensive, they will usually bite anything that catches or touches them, but can be safely handled, either by catching them firmly in the hand from above or by letting them run over the hands or arms or from one hand to the other. They are not easily so tamed as to be gentle and safe to play with, but, unlike many of the native mice of other groups, are not timid and nervous.

#### INDIVIDUALITY

There is great individuality, however, in dispositions and habits, some mice being comparatively timid and nervous, while others are more gentle and confiding, seeking rather than avoiding notice. Some are

very fond of certain foods which others have not learned to like or will not eat. Some individuals are very talkative, always squeaking or making some noise at the others, while more of the individuals are quiet and generally silent. Some are far more pugnacious than others and often bite if handled, while others may be held in the hands and stroked without offering any resistance. These differences are noticed in the young just beginning to run about, and seem to be to some extent inherited characteristics. Certain pairs will live together in perfect accord, while others will quarrel and fight and refuse to occupy the same nest. Some females will accept the attentions of only one male and will savagely resent the advances of all others, while others show no preference and will accept attentions promiscuously.

Most of the males are highly polygamous, but one pair that lived together until after the young were born were from the first very affectionate, remaining much of the time together in the nest box and paying little attention to the mice in neighboring cages. This male would not voluntarily leave his cage nor enter the cage of another female, even when called. This was very unusual, as most of the males were eager to go into other cages and make friends with the females or fight with the males.

In No. 6 cage a male and female had lived contentedly together and raised two families of young, while against their cage an old female lived alone in No. 3 and was on friendly terms with her neighbors through the wires. After she had given birth to her fourth family of eight young she was making her peculiar squeaking calls, which the male in the other cage evidently recognized as an invitation to visit her, and as he eagerly came up over the edge of his cage and down into hers the female in cage No. 6 became greatly excited and twice forced her way out under the roof of her cage and tried to get into cage No. 3 to punish her rival. Not being allowed to do this she stormed around her own cage, squeaking, kicking up the sand on the floor, biting the wires, and showing every indication of rage until her mate came home in the evening. Then she pounced upon him, bit his nose, chased him around the cage, squeaking and scolding at him until he was severely punished. She was cross with him all the evening, but the next day had settled down to her usual pleasant frame of mind and had evidently forgotten her domestic troubles.

A neighboring male that came into cage No. 1 during the mating time of the old pair that had been captured in their wild state was pounced upon by the female and his foot so badly bitten that he was glad to escape to his own cage and nurse his bloody paw. In other and the majority of cases the females made no objection to their mates visiting other females as much as they wished, and these two cases are exceptions to the usual free-love manner of life among these polygamous little animals.

These incidents, however, show variable tendencies in the social life of the meadow mice, which, under certain conditions of environment, might lead to perfect monogamy if for any reason this should prove beneficial to the species.

#### PLAYING

With such quick and energetic little animals it is sometimes difficult to distinguish between work and exercise and mere play. The young, soon after their eyes are open, will push and pull and roll each other about in unmistakable sport and up to half-grown size are conspicuously



playful, but the vigorous exercise of the older animals seems more like the necessary release of superabundant energy, or sometimes an expression of nervous excitement. In the cages they get abundant exercise and apparently much enjoyment from running in their hollow revolving wheels or around on the inclined disks, sometimes two running side by side in a wheel, or two to five running together at high speed in perfect time and step on a rapidly revolving disk. They soon acquire great skill in jumping off and on without checking the motion of the disk, and if the motion is checked they may all reverse and start at full speed in the opposite direction. In a cage with a large number of occupants the wheel is kept going much of the time both night and day, even by old and fully grown animals, but with one or a pair in a cage, the early morning and evening are the busy hours for exercise, or play, as you choose to call it.

#### FIGHTING

In their own families and among their own friends meadow mice are generally friendly, playful, and even affectionate, but there are times when with strangers, rivals, or intruders they are vicious little savages. The mother will fight anything from another mouse to a bulldog or a man in defense of her young. Possession of a cage, a nest, or a favorite corner is sometimes the cause of a quarrel, of squeaky disputes, or even a fight in which the intruder generally yields and retires. The mating of a strange male and female often begins with several little fights in which the male sometimes gets a bloody nose, while the female seems never to be injured, but usually after a few minutes they are fully mated and the affair has passed as a mere formality of microtine etiquette. If the female is not ready for mating she may keep up attacks on the male until he is forced to retire to a corner and nurse his injured feelings, if not an injured nose.

The real fights are among rival males and usually males from different families, and these are apt to be serious or sometimes fatal. One nearly full-grown male that by mistake was dropped into the cage with an older and larger male was nearly killed in a few minutes before it could be rescued. Both fought savagely and so fast that their motions were a complete blur, so that nothing could be seen of their methods of attack. When separated, the smaller one was found to be so badly injured that he was killed and his skin and skull saved for a specimen. He was bitten around the head, on the back, belly, feet, and tail, two large gashes were cut across the abdomen, and the skin of the back was so full of holes that it looked like a shotgun target. He would have been killed in a very short time if left in the fight. The larger male seemed stiff and sore for a few days, but recovered from his injuries. Occasionally, a male is trapped in the meadows with his skin cut full of holes from a fight, in which case he may be safely assumed to be the sole survivor of a fatal combat.

#### SANITATION

Like most wild animals, meadow mice are as neat and cleanly in habits as circumstances permit. Although often digging in the earth and running through the mud they also run through shallow water and swim and dive on occasion. Their fur is fairly waterproof and when wet quickly dries as it is carefully combed and cleaned. After working in the earth the mice clean their nails and comb their fur, which they

usually keep in perfect condition. Without plenty of water or dry earth the fur becomes oily and rough, but either water or sand removes the excess oil and leaves it light and fluffy.

The nests are kept clean while in use and are abandoned when they get old and stale. New nests with soft linings are usually prepared a few days in advance for each litter of young, so that new nests or fresh linings are generally provided about every 20 days.

Their little long green pellet-like excrements are more or less scattered along the trails and on the feeding grounds, but in the burrows or near the nests they are deposited in little heaps in out-of-the-way places. In the cages some corner farthest from the nest is usually chosen for the purpose, or sometimes the water dish is used as a urinal and the pellets are deposited in a corner or in another dish. In such case to keep the cages from becoming foul they have to be cleaned frequently and supplied with fresh sand or sawdust. As an experiment, outside receptacles were provided by placing a square tin can with a hole in the side large enough for the mice to enter from a corresponding hole in the side of the cage. An inch of sand in the bottom of this can gave an attractive little dark compartment which the meadow mice at once adopted and used exclusively for toilet purposes. As the cans merely hooked onto the outside of the cages and could be conveniently emptied and supplied with fresh sand every day or two, the cages were kept clean with little trouble.

#### BREEDING HABITS

It has long been known that these mice are very prolific breeders, specimens of females containing embryos, usually four to eight in number, being taken at all seasons. The mammae are arranged in four pairs, two of inguinal, and two of pectoral on four elongated mammary glands, and eight young should be the normal maximum number.

#### FREQUENCY

In captivity the young, if well fed, begin to breed when less than half grown, the females mating with older males when only 25 days old and having young when 45 days old, and the young males mating when only 45 days old. The period of gestation is approximately 21 days, in one case 20 days and 17 hours. The breeding activities are practically continuous, the females mating immediately after the birth of the young and producing litters of usually 4 at first, but when full grown, after the first or second litter, usually 6 or 8 at a birth. Seventeen consecutive litters of young have been produced by one female in captivity within a year—May 25, June 14, July 8, July 29, August 23, September 18, October 18, November 9, November 30, December 21, January 12, February 2, February 23, March 18, April 8, April 30, May 20—and then she showed no signs of being near the end of the breeding period, while several generations of her young have busily followed her example, one of them, born on May 25, having produced 13 families of young, totaling 78 in number, before she was a year old.

The number of young in a litter ranges from 2 to 9, and with the original pair, averaged 5, totaling 83 in 17 litters. At this rate of increase, allowing equal numbers of males and females and the young beginning to breed at 46 days old, the total increase from one pair, if all lived and bred, would be over 1,000,000 individuals at the end of a year. If all

these were confined to one acre of ground, this would mean a little more than 20 mice to every square foot.

#### MATING

"Mating" is only for the immediate needs, and a misnomer at that, because a female usually accepts the attention of any number of males in rapid succession and shows no choice of individuals, favor to young or old, or any regard to relationship, whether sire or brothers or previous offspring. There seems to be no moral necessity of life with them other than the most rapid increase possible of individuals of the species. The one exception to complete promiscuity is provided by nature in the slower sexual development of the males, which prevents inbreeding within the litter before the young have scattered, the males not coming to sexual maturity until about the time the first young of the females of the same litter are born. Furthermore, respect for the females during pregnancy by even the most virile old males is enforced with such spirit that it seems never to be questioned.

When a half-grown female jumps and squeaks at an old male, he cringes or runs, for he knows that her teeth are sharp and that she will not hesitate to use them. If he comes too near her newly born young, the mother will sometimes punish him severely before he can get out of reach. If the very young are left unguarded, the males or other members of the family will often kill and eat them or sometimes wantonly bite and kill them all before their mother returns. In crowded cages it is difficult to prevent the young from being killed, but the mother is a model of care and solicitude, ready to fight to the last for them and to use all her intelligence and energy in their protection.

#### NESTS

The female builds a new nest for the reception of the young, endeavoring to place it where none can find it, but if discovered and disturbed she promptly removes the young to a place of safety. If suddenly alarmed she often rushes out with all of the young clinging for dear life to her nipples and drags them for some distance along the runway or under cover, where she can gather them and carry them one at a time in her mouth to another nest. So quick and shuttlelike are her motions that the young disappear as if by magic, and when one nest box is disturbed the young will all be found a few moments later in another.

In cold weather the nest is built especially thick and warm, and the newly born young are not left for more than a few moments at a time, and then the mother covers them up securely before she leaves them.

One old mother in No. 3 cage had a new family one cold night in March. In the morning she was very hungry for her breakfast when it was placed in the cage, but was greatly worried about the young getting cold if she left them for a minute. She rushed out of the nest box and back several times without stopping to eat, and seemed greatly disturbed. Suddenly she rushed up to her one remaining young of the previous litter, then 22 days old, well furred and nearly half grown, seized it in her mouth and carried it into the nest, and leaving it with the tiny naked babies, returned to the food. The foster mother soon reappeared at the breakfast table but was instantly grabbed up, rather roughly, and with many squeaks carried back into the nest, where this time it remained while the real mother finished her breakfast.

## CARE OF YOUNG

The young are hairless and weigh only about 3 gm. each when born, with closed eyes and ears and no trace of teeth. They grow rapidly, however, gaining after the first few days about 1 gm. a day until over half grown. Their dark-colored fur begins to appear as soft velvet in five or six days, their incisor teeth about the fifth, the molar teeth about the seventh, and their eyes and ears open on or near the eighth day. As soon as their eyes are open they are quick to run and hide if disturbed, and a few days later are out of the nest searching for food, following the trails, and, if in cages, running on the wheels, playing and pushing for their rights.

When about 12 days old, the young are weaned, but they remain with their mother, occupying the old nest and holding together in friendly family relations until time for the next installment of young, when the mother seeks or builds a new nest and leaves her previous family to care for itself. If food were abundant, they would remain contentedly together for an indefinite time but for the disturbing sexual forces which before they are full grown impel the females to seek new homes for prospective offspring and the males to wander constantly in search of one mate after another.

## FACTORS MODIFYING BREEDING

Factors modifying breeding activities are food, weather, cover, proximity, and contentment. A group of 9 mice, 5 females of breeding age and 4 males, kept in roomy cages and starved for 36 days until hungry, thin, cross, and squealing like half-fed pigs, showed no signs of breeding until at the close of the period when the quantity and quality of their food had been brought up to a satisfying point. The females, fighting the males away, kept them constantly cowed and in fear of their lives and actually killed and partly ate one old male, the largest animal in the cage. They also killed and ate one quarter-grown young at the time when famine was sorest. As their food was increased and changed to more nutritious quality the females rapidly yielded to male attention and all were quiet and contented again. Even for this short period, others in cages where well fed had raised one family of young and had the next well under way. It seems highly probable that a natural shortage of food in a dry year or some other cause might retard breeding for a much longer or for an indefinite period. The three litters of young afterward born to the starved females numbered only 3, 4, and 5, while at the same time, in cages well supplied with food, 5, 6, and 8 were the usual numbers.

Weather apparently has an effect on the rate of breeding, for while under certain conditions the mice breed in winter, their breeding activities are generally much retarded then or entirely suspended. Of great numbers of specimens collected at all seasons of the year, few females are found to contain embryos in winter except in warm southern climates or where there is unusual protection. In Minnesota tiny naked young are sometimes found in nests under haystacks in midwinter, but in less sheltered localities with only the normal winter food they are rarely found. While the reduced winter food supply may be partly responsible for the general suspension of winter breeding, the excessive cold seems undoubtedly a potent factor. The continuous hot weather of the Tropics

seems to be a bar even to the existence of the mice, but in the Temperate Zones they breed continuously through the warm seasons.

Cover, or some secure place in which to construct the nests, seems necessary for favorable breeding conditions or for the contentment of the animals. In fact, where not protected from overhead enemies by some concealing screen of vegetation or other cover they are soon exterminated.

Scarcity of individuals may often tend to retard or entirely to check reproduction. The mice may be left stranded out of reach of mates, when breeding perforce ceases until stimulated by a gradual influx from other localities.

Discontent with environmental conditions, nature of soil, lack of water, terror of enemies, or any other disturbing elements might well prevent breeding and scatter the individuals. A change from an old cage to a new and better one will sometimes cause several days of unhappiness, struggle and effort to escape, gnawing at the wires, frantic running round and round the walls, impatience and snappishness with each other, and especially a savage attitude of females toward the males.

On the other hand, breeding is evidently stimulated by rich food, mild weather, safety, quiet, sociability, comfortable living conditions, and general contentment. How far this would continue without degeneration of the vigor of the species has not been determined, but for a time at least the stimulating effects of abundance and prosperity are pronounced.

#### FOOD HABITS

In their native habitat meadow mice feed to a great extent on grasses and sedges, selecting the tender new shoots in spring, the inner hearts at the base in summer, the green and ripening seeds in summer and autumn, and the tender bases, dormant sprouts, roots and root stalks, bulbs, tubers, and the bark and buds in winter. They feed also on the clovers and a great variety of meadow and upland plants, eating both the green tops, flowers, and seeds, and the roots.

In times of scarcity, especially in winter and under protection of snow, the mice eat the inner bark and tender cambium layer from shrubs, trees, and vines, often completely girdling the trunks as high as they can reach above ground and stripping the roots well below the surface.

In fields, gardens, orchards, and home grounds the mice show a fondness for grains of all kinds, most garden vegetables, and the bark of many fruit and ornamental trees, shrubs, and vines. Often they cut down and destroy and waste far more growing vegetation than they eat.

#### STORES

In times of abundance, and especially in autumn, food is stored for future use. Along the upper Missouri for ages Indian tribes have gathered part of their winter food by robbing the mouse stores of ground beans, artichokes, and other tubers. Several quarts or a peck of these beans and tubers are often taken from a single cavity, and a person can sometimes gather several bushels a day from the mouse stores (3). In other parts of the country various roots, tubers, and bulbs are stored. In captivity the mice will often secrete all surplus food under or near the nest, sometimes filling their nest boxes full of seeds, grains, and vegetables. In cold weather when the young are about to be born, the mother stores up all spare food and places it around the nest where it can be

reached without her leaving the delicate, naked young or exposing them to the cold. Approaching maternity may often be noted by this habit.

#### IN CAPTIVITY

In captivity mice will eat a great variety of green vegetation, but they show an especial fondness for all of the clovers and other legumes. Grass is extensively eaten, but does not satisfy them so well as clover, and is always left if abundance of both are fed together. Mice are especially fond of cantaloupes, completely devouring the rinds left from the table. Green corn is one of their favorite foods, and even the cobs are picked bare to the core. Most vegetables are eaten with more or less relish, as well as all grains and seeds, bread, fruit, fresh meat, fat, or bacon. Rolled oats seems to be a favorite food with both young and old.

Like all their vital processes, feeding and the elimination of waste are very rapid. The animals apparently eat every few hours all day and all night, and the accumulations of pellets in the corners of the cages grow rapidly.

#### QUANTITY OF FOOD REQUIRED

The quantity of food eaten is astonishing. In one cage, 30 days' feeding of 10 mice with all the clover, cantaloupe, grain, and seeds they would eat showed, after deducting 10 per cent for waste which could not be otherwise accounted for, that an average of 55 per cent of the weight of each animal was eaten every 24 hours. This was on the richest kind of food, such as they rarely obtain in the wild state.

In another cage during the same period nine that were fed grass, clover, and cantaloupe rinds, with no grain or seeds, ate, after deducting 10 per cent for waste, an average of 107 per cent of their weight every 24 hours. This would seem more nearly their normal ration in a wild state and the best basis for computing food consumption. Some days they ate nearly twice their weight in green food, but only after they had become unusually ravenous. In both cages they had revolving wheels on which they exercised vigorously and were living fairly normal, contented lives.

In one cage the average weight of the animals was 30 and in another 33 gm. a part of the animals being immature. The adults average about 50 gm. but 30 gm. would be a fair average for the general run of young and old meadow mice in the field.

#### AGGREGATE DESTRUCTIVENESS

At 30 gm. a day one meadow mouse would consume 10,950 gm. (23 pounds) of green food in a year, and 100 mice 2,300 pounds, or a little over a ton of green grass or clover, which would make about half a ton of dry hay.

A hundred mice to an acre is not an unusual number in meadows favorable to their habits, while in "mouse years" or during mouse plagues, the number has been estimated at thousands to the acre. Even with 1,000 to the acre it is easily shown that mice consume more vegetation (11½ tons) than would ordinarily grow on an acre in a year.

Mouse plagues, disastrous as they are locally, are of minor importance in comparison with the steady yearly drain on crops by the mice over the country at large in normal years. Even as few as 10 meadow mice to the acre on 100 acres of meadow would take about 11 tons of grass, or 5½ tons of hay, a year. Or this number on the 65,000,000 acres of hay raised in the 38 mouse States [see "Monthly Crop Reporter," p. 153, 1921 (21)], would cause a loss of over 3,000,000 tons of hay a year, or a money loss of some \$30,000,000 annually in hay alone.

Of other crops, grains suffer most from the ravages of meadow mice, especially when left long in shocks or stacks after harvest and in situations where accessible, but from the seed and sprouting grain to the ripened crop all grains are eagerly eaten. Small fields are likely to suffer most, as the mice penetrate less readily to the middle of large cultivated areas.

Root crops and vegetables are sometimes injured by meadow mice, but usually not so seriously as by the more exclusively burrowing pine mice (*Pitymys*). In times of great abundance of the mice, however, they have been known seriously to injure the potato crop and almost to destroy many garden vegetables.

Destruction of fruit trees and many ornamental trees and shrubs in winter by the mice eating the bark from the base of the trunks, often completely girdling and eventually killing them, causes more annoyance than any of the more serious and less conspicuous losses. This is usually done under cover of deep snow with no indication of harm until the snow has disappeared in spring. Many fine orchards have thus been seriously injured or completely ruined.

#### METHODS OF CONTROL

The importance of keeping these mice under control and at a minimum number is clearly seen. Much has been published on their habits, depredations, and the most economical methods of destruction, and the appended references are to papers that cover this phase of the subject: 4; 5; 9, p. 97-102; 10; 11; 12; 16; 18, p. 97-201; 19, p. 515-537; 20.

The most economical and practical method of control, that is, control by natural enemies, has been given least emphasis. In his studies of the food of hawks and owls (7, 8), Dr. A. K. Fisher has shown the great value of these birds in reducing the numbers of meadow mice, but the value of many other birds which feed upon them—gulls, terns, herons, bitterns, cranes, ravens, crows, magpies, shrikes, jays, and others—has not yet been fully recognized. The enormous consumption of these mice by skunks, minks, weasels, martens, badgers, foxes, coyotes, bobcats, lynxes, and even bears can not fail to have a marked influence on their abundance. To what extent they are preyed upon by shrews and flesh-eating rodents is not fully known. Snakes and even fish help to keep them under control.

The mouse problem involves more than protecting the enemies that are otherwise not too obnoxious; it means giving these enemies a chance to see and catch the mice, at least at the borders of the fields and meadows, and along the roadsides. Simple cultural methods, that would pay for themselves in the reduction of weeds and in added beauty of landscape, would remove the permanent cover that makes it possible for the mice to persist in otherwise well-cultivated areas. Clean fields and meadows, and clean borders, roadsides, and ditch banks, would help to solve the

problem of mouse control, prevent occasional heavy losses, and add considerably to the yearly farm returns.

### USES

Total extermination of meadow mice, even locally, would be as impossible as it would be undesirable. These rodents are firmly entrenched in the waste places of the earth where they do no harm and may do some good. Then, too, they have a value, even from our narrow and selfish point of view. Seton has called them "the boats especially designed to bring over food from the Mainland of Grass to the Island of Carnivores," and they certainly are largely instrumental in supporting many of the animals that provide us with warm furs. They are also the daily bread of numerous birds of prey that agriculture could not well spare without great danger from other rodent pests. To some of the native tribes of America they still supply an important part of the winter vegetables from their winter stores of ground beans, tubers, and roots (3).

More than any other group of small mammals, meadow mice hold the key to the balance of natural adjustment for a large portion of our native bird and mammal population. They have also a vivid lesson for us in the struggle of life, the surge of dynamic biological force against the shores of relentless destruction, a fierce struggle for existence, which, through highly perfected adaptation to environment, has won a well-balanced coordination of body, mind, and morals that serves the best end for the perpetuation of the species.

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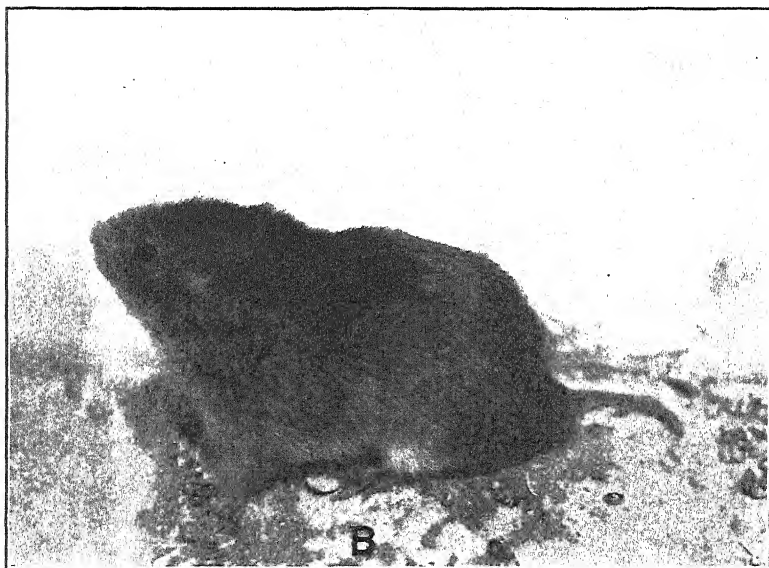
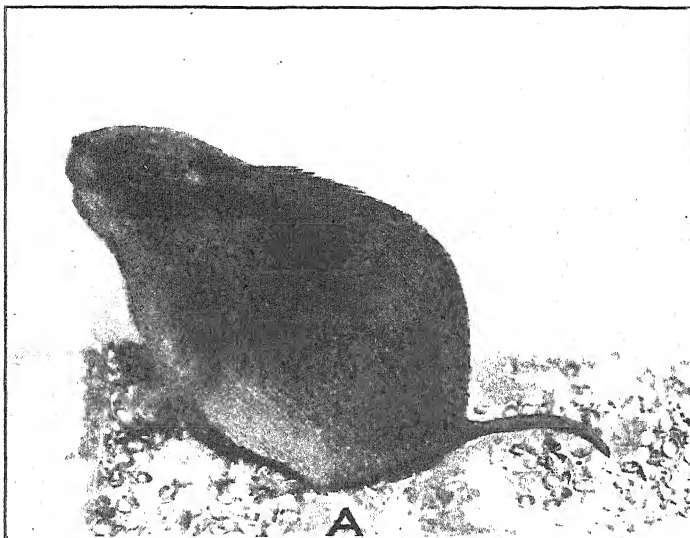


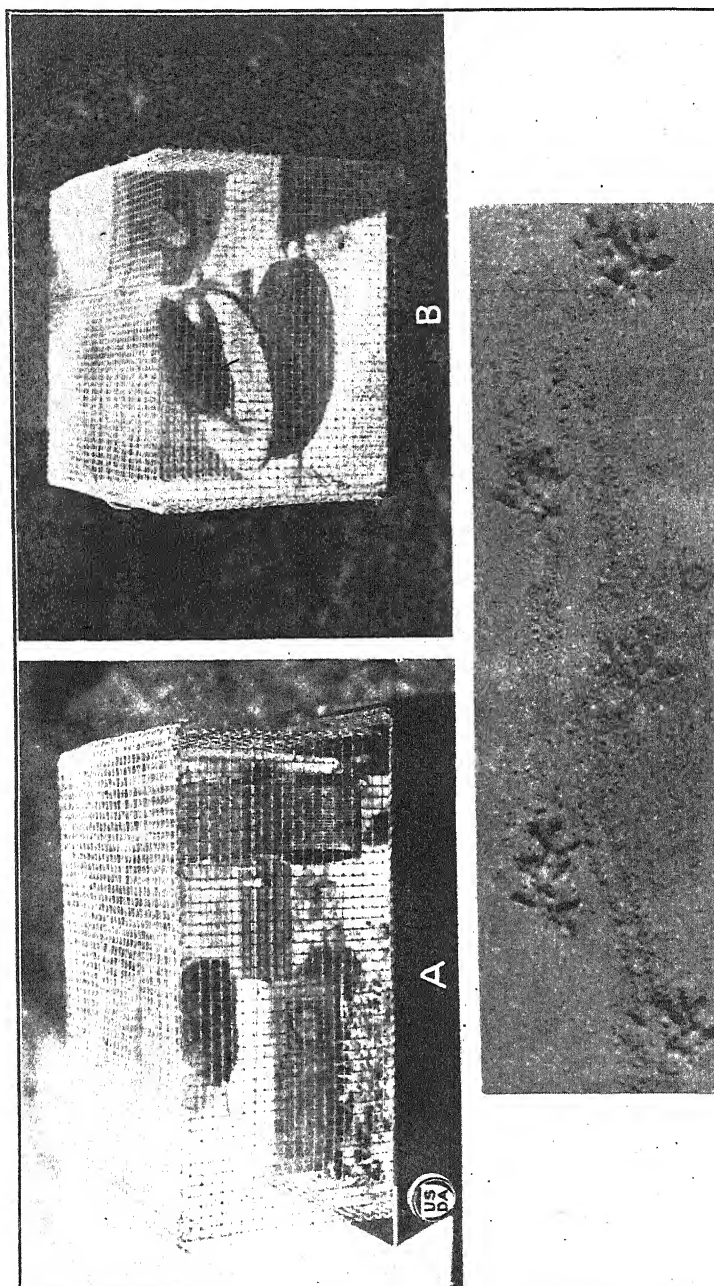
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PLATE I

A.—The original female used in the studies described, weighed when captured, April 1, 1922, 32 gm. On August 20, just before the birth of six young, she weighed 70 gm., and after their birth 54 gm. On May 20, 1923, just before the birth of six young she weighed 105 gm., and after the young were born 80 gm.

B.—The old male when captured, April 1, 1922, weighed only 36 gm. On August 6 he weighed 56 gm., and on May 20, 1923, 72 gm. Another male born on May 25 weighed when a year old 81 gm.





## PLATE 2

A.—A simple wire-mesh cage in a sand pan, with nest box, food and water dishes, and a vertical hollow wheel for exercise, makes comfortable quarters for mice and is easily cared for. The top is hinged so as to open upward.

B.—A small, quarter-inch wire-mesh cage containing nest box and water dish on shelf, a revolving disk for exercise, and sand and food dishes on the floor, will accommodate one mouse very comfortably and can be connected by side doors with other cages.

C.—Tracks of a meadow mouse trotting over a thin layer of white flour; about natural size.

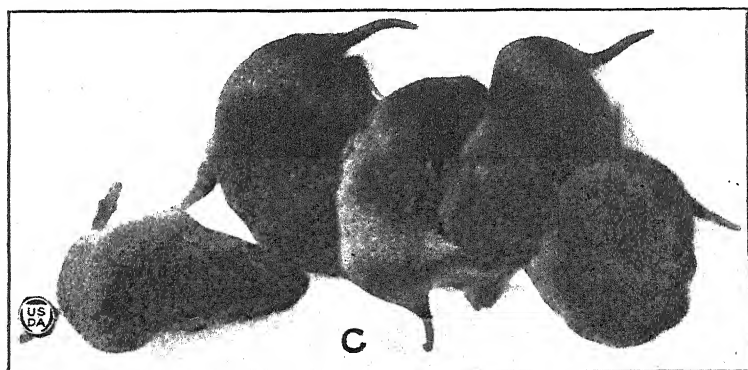
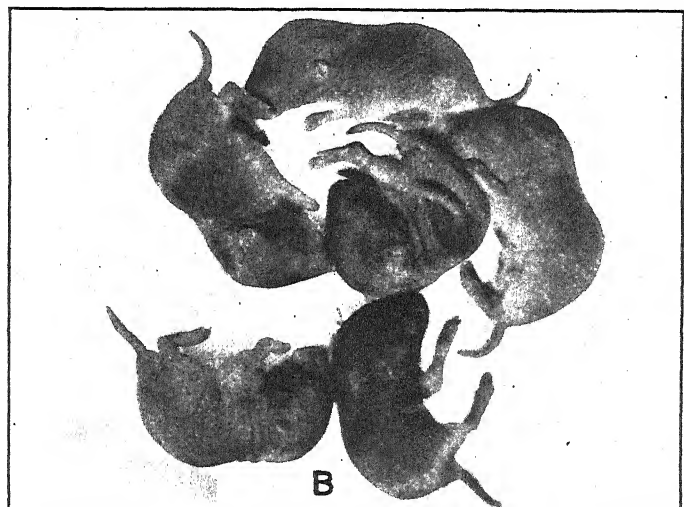
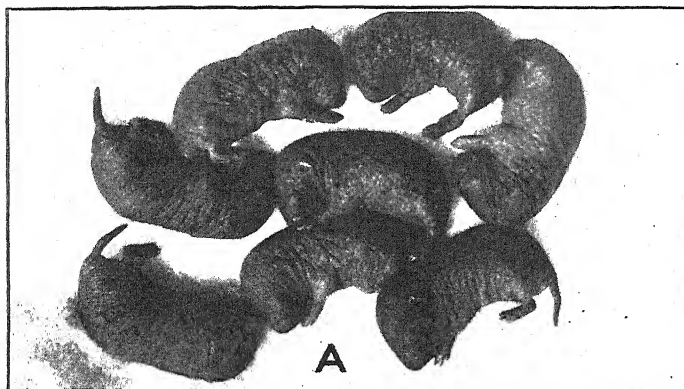
PLATE 3

A.—Young just born.

B.—Young four days old.

C.—Young seven days old just before the eyes and ears open.

The young at birth are naked, blind, toothless, and helpless, weighing only about 3 gm. each, but they grow rapidly. All about natural size.







# INHERITANCE OF THE CRINKLY, RAMOSE, AND BRACHYTIC CHARACTERS OF MAIZE IN HYBRIDS WITH TEOSINTE<sup>1</sup>

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## INTRODUCTION

To be of maximum efficiency, plant breeding must deal with all existing wild relatives of the plant being bred, for it is only in this way that investigators can take advantage of special characteristics acquired through ages of evolutionary progress. *Zea* is a monotypic genus, and its nearest wild relatives are the two species of large Mexican grasses, *Euchlaena mexicana* and *Euchlaena perennis*, commonly known as teosinte. As a first step in ascertaining the possibilities of combining the desirable characters of maize and teosinte it is essential to determine the hereditary behavior of hybrids between them. Progress in this direction was reported by Collins and Kempton in the analysis of a hybrid between Tom Thumb pop corn and *Euchlaena mexicana* (5).<sup>2</sup> In this hybrid there was seemingly complete blending of maize and teosinte characters; it became of interest, therefore, to analyze the behavior of some of the more strictly Mendelian variations of maize in hybrids with teosinte. The present paper reports the inheritance of three striking Mendelian characters of maize, crinkly, ramose and brachytic, in hybrids with the annual teosinte, *Euchlaena mexicana*. The inheritance of these three variations in maize hybrids is now fairly well understood, all three behaving as simple Mendelian characters recessive to the normal form.

The great variability of maize has become a byword. Each year sees the discovery of a new series of true breeding teratological forms, embracing almost every part of the plant, leading to the belief that the number of different sorts is infinite. In fact, so numerous are abnormal forms that improvement in maize has become centered largely on the problem of eliminating deleterious mutations from existing varieties.

In view of this abundance of variations in maize it seems rather surprising that its nearest wild relative, *Euchlaena mexicana*, is unusually free from true-breeding abnormal variations. Although more self-fertilization probably occurs in *Euchlaena* than in maize, there is enough cross-fertilization to permit degenerative recessive variations to survive as heterozygotes, so that the difference in stability hardly can be ascribed to the relatively greater homozygosity of *Euchlaena*.

Crosses between Florida teosinte, *Euchlaena mexicana*, and most of the well-known Mendelian variations of maize are being made as opportunity offers, as well as crosses between these variations and the perennial species *Euchlaena perennis*. The two species of *Euchlaena* seem to react differently in hybrids with maize, and the first generation

<sup>1</sup> Received for publication Sept. 8, 1923.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 596.

of hybrids with the perennial species shows a much higher degree of dominance of all the characters of teosinte than has been found in hybrids with the annual species.

At the present time,  $F_2$  populations have been raised from crosses of *Euchlaena mexicana* with golden (13), ramose (9), brachytic (10), rainbow (1), and crinkly (7), while  $F_1$  progenies have been grown with dwarf (6), and pod (14).<sup>3</sup>

In making these hybrids teosinte almost invariably is used as the female parent, since in controlling the flowering stage of this species by shortening the length of day, the staminate flowers are suppressed. As teosinte has but from 7 to 10 seeds in each pistillate spike, only a few seeds are obtained as the result of each pollination; the first-generation populations therefore are small. In the crosses with crinkly, ramose, and brachytic maize, however, teosinte served as the male parent; with the crinkly an unusually large number of seeds were obtained, in consequence of which a large  $F_1$  population of this hybrid was grown.<sup>4</sup> The hybrids differ somewhat in general vigor and in the size of the plants, one of the most vigorous being the crinkly cross, which seems to have commercial possibilities as a forage plant. In the character of the inflorescences, all resembled the hybrid with Tom Thumb, with the exception of the cross with the dominant variation pod. This, as was to be expected, produced long glumes on the pistillate spikes similar to those of a podded ear. These long glumes on the characteristic four-rowed ear of the  $F_1$  maize-teosinte hybrid give a rather startling resemblance to a gigantic wheat head (Pl. i).

The hybrids with golden and rainbow, of course, were normal with respect to these characters, and segregated in the  $F_2$  into numerous grades of the parental colors.<sup>5</sup> In a population of 371 plants of the rainbow cross no plants as fully variegated as the maize parent were recovered, though many truly beautiful plants were produced.

In the hybrid with golden, plants were obtained fully as yellow as the parent, if not more so, though there was no clear distinction between golden and green. The segregation in the  $F_2$  of these hybrids suggests that *Euchlaena* possesses modifying factors for both of these characters and, as in other hybrids, the form of inflorescence seemed to be associated but loosely with the Mendelian characters under observation.

The inheritance of the other three characters, crinkly, ramose and brachytic, is capable of quantitative analysis, which should offer more critical evidence on the existence in *Euchlaena mexicana* of modifying factors for these characters.

<sup>3</sup> Seed stocks of golden and crinkly were received from R. A. Emerson, ramose from W. B. Gernert, pod from H. S. Sconce, while rainbow was purchased from Luther Burbank and brachytic and dwarf arose in our own pedigreed stock. The degree of yellow in the original golden strain was somewhat variable, but the strain finally used in the cross with *Euchlaena* was developed from the original stock with rather an extreme expression of the yellow pigment. The stock of rainbow purchased from Burbank has well-developed white and yellow stripes, together with the red plant color. The inheritance of the japonica striping has been reported by Lindstrom (12), while that of red plant color has been reported by Emerson (8). The stock as originally received was heterozygous for red plant color and for yellow as opposed to white striping. The degree of expression of all of these characters was variable, though no self-colored plants have been found in the original stock. Through selection and inbreeding it has been possible to isolate lines having almost chlorophyll-free upper blades, and others practically self-green. The seedling leaves of these divergent strains are very similar, perhaps the only difference being the rather earlier appearance of variegation in the extreme white strains.

In the cross with *Euchlaena* a medium-variegated plant was used, being homozygous for red plant color and heterozygous for white and yellow stripes.

<sup>4</sup> These crosses were made by C. G. Marshall at Chula Vista, Calif., and the  $F_1$  and  $F_2$  populations were grown under his direction at the same place.

<sup>5</sup> The  $F_1$  plants of the hybrid with rainbow were red, as was to be expected, but were normal in that the striped condition of rainbow was completely recessive.

The fact that these teratological characters can be recognized in a homozygous condition permits measuring their correlations with other more complex multiple factor characters, and thus furnishes an opportunity to determine to what extent they are correlated with the characters that differentiate maize from teosinte.

The hybrid between Tom Thumb pop corn and teosinte showed that the characters of maize were correlated only to a very slight degree and that in many cases there were definite disherences where characters derived, one from the maize and the other from the teosinte parent, tended to reappear together. With the numerous factors involved, largely in a heterozygous condition, only low correlations could be expected; but with simple or comparatively simple characters which can be recognized in the homozygous-recessive condition one would expect relatively higher, though fewer, correlations with the characters which differentiate maize from teosinte than were found in the Tom Thumb-teosinte hybrid.

#### CRINKLY $\times$ TEOSINTE

Crinkly is a variation of complex constitution affecting the form of leaf, the length of the leaf sheath, the stature of the plant, and the form of the terminal inflorescence. In hybrids with maize it has been found possible to separate this complex of characters into its several component parts. As stated by Emerson (6), "All of these characters are so variable, however, that some plants classed as crinkly do not show prominently one or other of them. Considering all leaf characters together with stature and form of tassel, it is usually easily possible to separate crinkly from normal plants, but occasionally the separation is somewhat difficult."

Typical plants of crinkly are of short stature and have compact tassels with relatively short branches and central spikes; the leaves are short and broad, usually folded and crinkled, and often with pronounced lobes at the base, while the sheaths are short and often fail to cover the nodes. In the hybrids with teosinte there also was a difference in color, the crinkly plants seemingly being of a much darker green than plants of normal teosinte. In extreme cases the upper leaves of crinkly plants fail to unroll; not unfrequently in these cases pistillate flowers develop in the tassel or, more rarely, an immature ear is produced in the place of the lowest tassel branch or in the axil of the uppermost leaf. The crinkly plant used as the female parent of the hybrid with teosinte represented an average expression of these characters and possessed none of the characteristics of extreme plants.

Two first-generation plants were grown in the greenhouse at Chula Vista, Calif., each producing several hundred seeds. Progenies from both of these  $F_1$  plants were grown separately in the field, as well as a population of the first generation. The  $F_2$  populations differed somewhat with respect to certain characters, thus precluding their combination in a statistical analysis without recourse to weighting factors; they therefore were analyzed separately.

With respect to the behavior of the crinkly character, the plants proved to be very much alike. The analysis of only one, therefore, is presented in detail.

An effort was made to get measurements of the various crinkly characters, such as length and width of leaf, stature, and dimensions of the various parts of the tassel, and, in addition, the plants were graded as to

the degree of crinkly leaves, degree of lobing, and degree of color, each on a scale of from 0 to 9. Aside from these grades, the plants were classified into normal and crinkly classes, with the result that  $28.1 \pm 2.1$  per cent were adjudged to be crinklies. The fact that the second generation plants fell naturally into two groups representing 25 and 75 per cent of the individuals seems conclusive proof that a single gene is largely responsible for the differentiation. Unlike most monohybrid characters, however, there is no single morphological characteristic that is predominantly affected by this gene. At maturity the best pistillate inflorescence was harvested, and the various notes designed to measure the maize and teosinte characters were recorded.

#### CHARACTERS MEASURED

In the analysis of this hybrid 39 characters were studied, of which the means, standard deviations and probable errors are given in Table I for both the  $F_1$  and  $F_2$  populations, and the frequency distributions for the  $F_2$  population are shown in figures 1 to 39.

#### DESCRIPTION OF CHARACTERS

1. HEIGHT.—The height of the main culm is measured from the surface of the ground to the tip of the central spike of the tassel, and the measurements are recorded in decimeters. The average height of teosinte is 23 decimeters, of crinkly maize, 12 decimeters, of ramosa maize, 18 decimeters, of brachytic maize, 9 decimeters.

2. HEIGHT OF TALLEST SUCKER.—The height of the tallest sucker is obtained and recorded in the same manner as that of the main culm. In teosinte the tallest sucker is as high or higher than the central culm, while in the strains of maize used in these hybrids, suckers are produced but rarely, and they seldom are as tall as the main culm.

3. TOTAL SUCKER HEIGHT.—The total sucker height is the summation of the heights of all the branches arising at or below the surface of the ground, and is recorded in meters. The total sucker height in teosinte usually is 20, while in the strains of maize used in these hybrids the total sucker height seldom exceeds one meter.

4. NUMBER OF SUCKERS.—The number of suckers is the total number of branches arising at or below the surface of the ground. The number of suckers in teosinte is about 14, and in the strains of maize used in these hybrids the average number of suckers is less than 0.5.

5. LEAVES ABOVE.—Leaves above the uppermost branch of the main culm. This character corresponds to the number of nodes above the ear in maize. It is the number of leaves between the terminal inflorescence and the uppermost lateral branch. In teosinte this is 1; in crinkly, ramosa, and brachytic maize 5 or 6.

6. TOTAL NUMBER OF LEAVES.—This character is the total number of leaves produced on the main culm from germination to maturity. About every fifth leaf is marked with small tags until the fifteenth, which is usually in good condition at maturity. Teosinte produces about 37 leaves, and the maize parents about 20.

7. LENGTH OF BRANCHING SPACE.—This is the length in cm. of the branched portion of the terminal inflorescence of the main culm. In teosinte this measurement is about 7 cm., in crinkly maize 15 cm., in ramosa maize 28 cm., and in brachytic maize 12 cm.

8. **LENGTH OF CENTRAL SPIKE.**—The length in centimeters of the terminal spike of the staminate inflorescence of the main culm. In teosinte this measurement is about 8 cm.; in crinkly maize 12 cm., in ramose maize 5 cm., and in brachytic maize 30 cm.

9. **LENGTH OF LONGEST TASSEL BRANCH.**—The length in centimeters of the longest branch of the terminal inflorescence of the main culm. In teosinte this measurement is about 10 cm., in crinkly maize 9 cm., in ramose maize 20 cm., and in brachytic maize 25 cm.

10. **LENGTH OF GLUME.**—Length of the outer glume in millimeters. The spikelet for this measurement was chosen from about the middle of the central spike. The glumes of teosinte are from 10 to 11 mm. in length; of crinkly maize 7 to 8 mm.

11. **NUMBER OF TASSEL BRANCHES.**—The number of branches, primary, secondary, tertiary, and upward, of the terminal staminate inflorescence of the main culm. In teosinte this is usually 23, in crinkly maize 17, in ramose maize 133, and in brachytic maize 36.

12. **ROWS IN CENTRAL SPIKE.**—The number of rows of alicoles on the central spike of the terminal inflorescence of the main culm. In teosinte there are invariably two rows of alicoles, while in maize the number is four or more.

13. **LENGTH OF LEAF.**—Length in decimeters of the longest leaf produced on the main culm. In teosinte this is about 12, in crinkly maize 5, in ramose and brachytic maize about 9.

14. **WIDTH OF LEAF.**—Width in centimeters of the longest leaf. The leaves of teosinte are between 6 and 7 cm. wide, of crinkly about 13 cm., of ramose and brachytic about 10 cm.

15. **WIDTH INDEX.**—The width of the longest leaf in centimeters divided by the length in decimeters and multiplied by 10. The leaves of teosinte are more than 10 times as long as broad, of crinkly about 3 times, and of ramose and brachytic 9 or 10 times.

16. **LENGTH OF SHEATH.**—Length in centimeters of the sheath of the longest leaf. The sheaths of teosinte are about three times as long as those of crinkly maize.

17. **POSITION OF LONGEST LEAF.**—The number of leaves above the longest leaf of the main culm. In teosinte there are about 15 leaves above the longest, in maize 8 or 9.

18. **LEAVES ABOVE BEST INFLORESCENCE.**—The number of leaves above the best inflorescence of the main culm. In these hybrids the entire branch is considered as the inflorescence. In maize this number would be the same as the "Leaves above," but in the second generation plants the uppermost inflorescence often is not the best, and in teosinte there usually are three leaves above the best branch.

19. **LEAVES ON BEST INFLORESCENCE.**—The number of leaves borne on the best inflorescence. The number of leaves forms a convenient way to count the number of nodes. The best branch of teosinte usually has 3 nodes, while the ear of maize is borne on a branch with 10 or more nodes.

20. **LENGTH OF BEST INFLORESCENCE.**—Length in centimeters of the best inflorescence produced on the main culm. The ear and ear stalk of maize are about one-fourth the length of the best branch of teosinte.

21. **LENGTH OF EAR STALK OF BEST INFLORESCENCE.**—The length in centimeters of the stalk of the best inflorescence. The length of the ear stalk of maize is about one-tenth that of the stalk of the best branch of teosinte.

22. LENGTH OF ♀ PORTION OF BEST INFLORESCENCE.—The length in centimeters of the pistillate portion of the terminal panicle of the best inflorescence. In teosinte this character would rarely exceed 5, while in the strain of maize used in these hybrids it would be about 20.

23. NUMBER OF BRANCHES ON BEST INFLORESCENCE.—The number of branches in the terminal panicle of the best lateral inflorescence of the main culm. In teosinte there would be from 3 to 10 such branches, in maize none.

24. ROWS IN TERMINAL SPIKE OF BEST INFLORESCENCE.—The number of rows of alicoles in the terminal spike of the best inflorescence. In teosinte there are 2, in the strains of maize used, 6 to 10.

25. POSITION OF BEST SPIKE.—The position of the best spike of the lateral inflorescence. If borne in the axil of the prophyllum of a lateral branch, the spike was recorded as 1, if borne as the terminal spike, as 2, if at some intermediate node as 3. In teosinte this would be 3, in maize 1. In the ramose hybrid these positions were recorded as 0, 1, and 2.

26. LENGTH OF BEST SPIKE.—Length in centimeters of the best single spike produced on the best lateral inflorescence of the main culm. In teosinte this length would be about 5 cm., in maize about 20.

27. NUMBER ROWS OF ALICOLES ON BEST SPIKE.—The number of rows of alicoles on the best spike. In teosinte the number of rows always would be 2, in maize 6 to 10.

28. NUMBER DOUBLE ♀ ALICOLES.—The number of double female alicoles on the best spike. In teosinte there would be no double female alicoles; in maize all would be of this type.

29. NUMBER SINGLE ♀ ALICOLES.—The number of single female alicoles on the above spike. In teosinte there would be from 5 to 10 such alicoles, in maize none.

30. NUMBER OF SPIKES IN PROPHYLLARY.—The number of spikes borne on the branch in the axil of the prophyllum of the best lateral branch of the main culm. In teosinte the number of spikes would be from 3 to 8; in the strains of maize used in these hybrids there would be no such branches.

31. DAYS TO POLLEN.—The number of days from planting until the first pollen was shed. The number of days would vary with the time of planting, late planting resulting in a shortened period. At the time these plantings were made teosinte required 184 days to anthesis, while the strains of maize used in these hybrids required 75 to 85 days.

32. DAYS TO SILK.—The number of days from planting until the first silks appeared. As with days to pollen, this period is influenced by the time of planting. In teosinte 178 days were required; in maize 80 to 90 days.

33. DAYS POLLEN TO SILK.—The number of days elapsing after the first pollen was shed until the first silks appeared. Teosinte is protogynous (the silks appearing before the pollen is shed) while the crinkly form of maize is proterandrous (silks appearing after the anthesis). In the first generation of the crinkly hybrid the plants were proterandrous by a mean number of days of 2.08, while the second generation plants were protogynous by a mean of 2.04 days; this is expressed in Table I as -2.04. Teosinte is protogynous by an average of 6 days, while maize is proterandrous by from 2 to 6 days.

34. DEGREE OF CRINKLY.—An estimate of the degree of wrinkling of the leaf blades. The estimate was made on a scale of 0 to 9, smooth leaves being recorded as zero.

35. DEGREE OF LOBING.—An estimate of the degree of lobing of the leaves; no lobes being recorded as 0, and the highest degree of lobing as 9.

36. DEGREE OF COLOR.—An effort to grade the plants as to the color of the foliage, light green or teosinte color being recorded as 0, and extremely dark green as 9.

37. ALICOLE INDEX.—The number of single female alicoles divided by the sum of the single and double female alicoles and multiplied by 100. In teosinte this index would be 100, in maize 0.

38. CENTRAL SPIKE INDEX.—The length of the central spike of the tassel divided by the total tassel length and multiplied by 100. This measurement is designed to show the relative length of the central spike. In maize it would be high, in teosinte low.

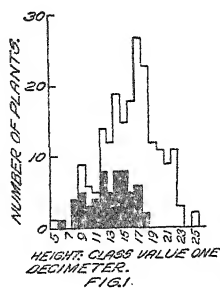
39. TASSEL BRANCH INDEX.—The length of the branching space of the tassel plus 10 minus the length of the longest tassel branch. This measurement is designed to show the relative length of the tassel branch. In maize this index would be high, in teosinte low.

TABLE I.—Biometrical constants for the first and second generations of the crinkly  $\times$  leosinte hybrid

Characters.	First generation.			Second generation.									Difference.	Big number of frequency polygon.
	Entire population.			Crinkly group.			Normal group.							
	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.					
Height.....	28.10	1.95	0.17	15.97	3.87	0.18	13.02	2.94	0.26	17.10	3.55	0.21	4.08 $\pm$ 0.33	1
Height of tallest sucker.....	26.80	2.19	.19	13.94	5.01	.24	11.24	3.75	.34	15.08	5.06	.30	3.84 $\pm$ .46	2
Total sucker height.....	12.69	3.45	.30	4.66	3.15	.15	3.02	1.95	.18	5.29	3.28	.20	2.27 $\pm$ .26	3
Number of suckers.....	5.86	1.57	.14	3.81	1.90	.09	3.07	1.20	.11	4.08	2.80	.17	1.01 $\pm$ .20	4
Leaves above.....	3.22	.64	.06	3.68	.97	.05	3.47	.93	.08	3.77	.96	.06	.30 $\pm$ .10	5
Total number of leaves.....	24.03	1.24	.11	23.06	2.08	.11	21.98	1.77	.16	23.50	2.08	.12	1.52 $\pm$ .17	6
Length of branching space.....	19.12	1.75	.16	11.97	2.94	.14	10.12	2.92	.26	12.32	2.96	.18	2.20 $\pm$ .32	7
Length of central spike.....	12.44	2.10	.20	8.73	2.97	.15	7.63	2.57	.23	9.14	2.96	.18	1.51 $\pm$ .28	8
Length of longest tassel branch.....	20.02	2.86	.26	13.16	3.74	.18	10.05	2.38	.21	14.45	3.48	.21	4.40 $\pm$ .28	9
Length of glume.....	10.68	1.33	.12	9.47	1.36	.07	8.98	1.16	.10	9.65	1.32	.08	.67 $\pm$ .14	10
Number of tassel branches.....	60.80	7.41	.67	43.17	13.60	.64	37.78	12.40	1.12	45.36	13.50	.80	7.58 $\pm$ 1.37	11
Rows in central spike.....	4.00	.00	.00	3.89	.73	.04	4.24	.47	.04	3.77	.72	.04	-.47 $\pm$ .00	12
Length of leaf.....	10.00	.87	.08	6.84	1.27	.06	5.54	.89	.08	7.40	.94	.05	1.86 $\pm$ .10	13
Width of leaf.....	8.98	.12	.11	7.79	1.78	.09	9.07	1.94	.18	7.27	1.35	.08	-1.80 $\pm$ .20	14
Width index.....	8.98	1.66	.15	11.96	4.47	.21	16.76	4.68	.42	9.94	1.84	.10	-6.82 $\pm$ .44	15
Length of sheath.....	18.36	2.18	.19	13.27	2.89	.14	10.25	1.73	.16	14.54	2.28	.13	4.29 $\pm$ .20	16
Position of longest leaf.....	9.33	1.02	.90	7.88	1.58	.08	7.47	1.27	.11	8.06	1.65	.10	.59 $\pm$ .14	17
Leaves above best inflorescence.....	5.26	.67	.06	4.72	1.00	.05	4.40	1.02	.11	4.86	.92	.05	.46 $\pm$ .10	18
Leaves on best inflorescence.....	5.59	.82	.07	5.56	1.29	.06	5.44	1.23	.09	5.60	1.32	.08	.16 $\pm$ .14	19
Length of best inflorescence.....	57.33	18.22	1.62	19.85	8.36	.41	15.09	3.77	.34	21.88	9.01	.53	6.79 $\pm$ .63	20
Length of ear stalk of best inflorescence.....	42.71	15.97	1.41	11.37	13.89	.39	7.44	3.64	.33	13.02	8.74	.52	5.58 $\pm$ .61	21
Length of $\frac{1}{2}$ portion of best inflorescence.....	1.07	1.80	.16	6.12	2.93	.14	6.84	1.95	.18	5.96	2.96	.18	-.88 $\pm$ .24	22
Number of branches on best inflorescence.....	10.50	3.32	.30	2.02	3.14	.16	1.09	2.26	.20	2.42	3.37	.20	1.33 $\pm$ .28	23



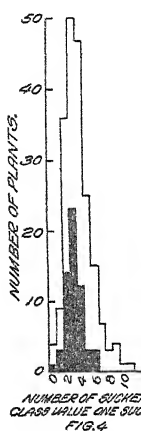
Rows in terminal spike of best inflorescence.....	3.97	.00	.00	3.57	.78	.04	3.66	.69	.06	3.53	.81	.49	-1.13 ± .49	24
Position of best spike.....	1.07	.36	.36	1.42	.63	.63	1.49	.62	.06	1.39	.64	.38	-.10 ± .37	25
Length of best spike.....	9.47	1.40	.13	8.02	1.58	.08	7.84	1.48	.13	8.11	1.61	.10	.27 ± .17	26
Number rows of alicoles on best spike.....	3.35	.83	.07	3.39	.97	.05	3.53	-.76	.07	3.32	.95	.57	-.21 ± .56	29
Number double ♀ alicoles on best spike.....	11.21	9.78	.87	19.80	20.70	1.04	30.44	21.65	1.95	15.62	18.60	1.12	-14.82 ± 2.24	27
Number single ♀ alicoles on best spike.....	24.93	16.58	1.48	18.59	14.10	.71	15.23	15.05	1.35	19.88	13.45	.81	4.65 ± 1.57	28
Number of spikes in prophyllary.....	4.76	1.35	.12	2.54	1.52	.07	1.96	1.01	.09	2.81	2.38	.14	.85 ± .17	30
Days to pollen.....	83.29	3.01	.26	94.01	9.65	.49	88.52	7.57	.68	96.16	10.04	.60	17.64 ± .90	31
Days to silk.....	85.37	4.15	.37	92.36	9.87	.48	86.41	7.44	.67	94.04	9.82	.59	18.23 ± .89	32
Days pollen to silk.....	2.08	1.86	.16	-2.04	2.03	.10	-2.28	3.04	.27	-2.24	2.84	.17	.03 ± .32	33
Degree of crinkly.....	.....	.....	.....	2.27	.87	.04	3.02	.73	.07	1.96	.73	.04	-1.06 ± .10	34
Degree of lobing.....	.....	.....	.....	.72	1.63	.08	2.41	2.25	.20	.05	.38	.02	-2.36 ± .20	35
Degree of color.....	.....	.....	.....	3.81	1.15	.05	4.86	1.14	.10	3.40	.86	.05	-1.46 ± .10	36
Alicole index.....	63.89	28.26	2.53	54.43	33.18	1.67	37.02	29.23	2.63	61.34	31.99	1.92	24.32 ± 3.30	37
Central spike index.....	.....	.....	.....	41.93	10.48	.54	41.70	12.45	1.29	42.00	9.74	.58	.30 ± 1.41	38
Tassel branch index.....	.....	.....	.....	8.58	3.45	.17	10.44	2.98	.29	7.95	3.37	.20	-2.49 ± .35	39



HEIGHT OF TALLEST SUCKER;  
CLASS VALUE ONE DECIMETER.  
FIG. 2.



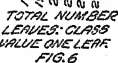
TOTAL SUCKER HEIGHT;  
CLASS VALUE ONE METER.  
FIG. 3.



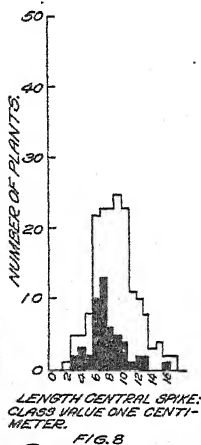
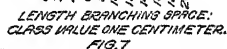
LEAVES ABOVE;  
CLASS VALUE ONE  
LEAF.  
FIG. 5.



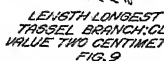
TOTAL NUMBER  
LEAVES; CLASS  
VALUE ONE LEAF.  
FIG. 6.



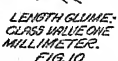
LENGTH BRANCHING SPACE;  
CLASS VALUE ONE CENTIMETER.  
FIG. 7.



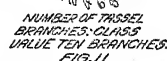
LENGTH LONGEST  
TASSSEL BRANCH; CLASS  
VALUE TWO CENTIMETERS.  
FIG. 9.



LENGTH GLUME;  
CLASS VALUE ONE  
MILLIMETER.  
FIG. 10.



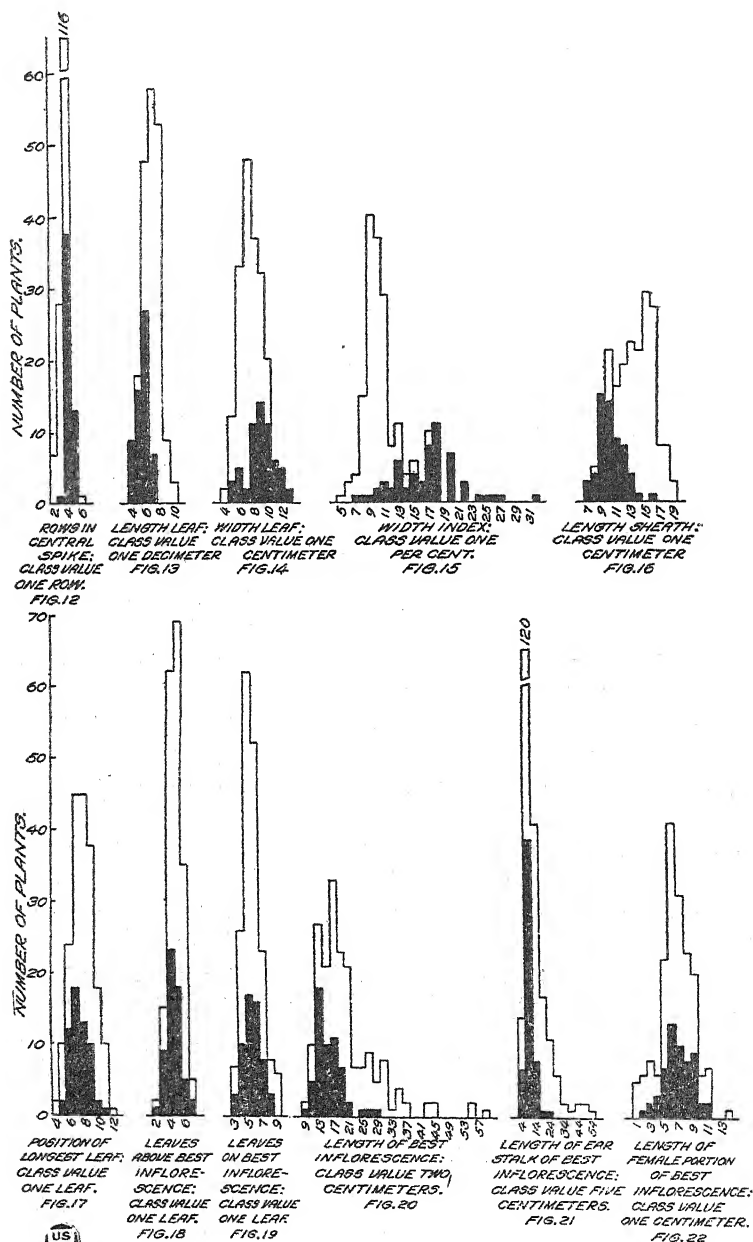
NUMBER OF TASSSEL  
BRANCHES; CLASS  
VALUE TEN BRANCHES.  
FIG. 11.



FREQUENCY DISTRIBUTION OF PLANTS IN  $F_2$   
SHADED PORTION REPRESENTS PLANTS CLASSED AS CRINKLY.

## FIGURES 1 TO 11

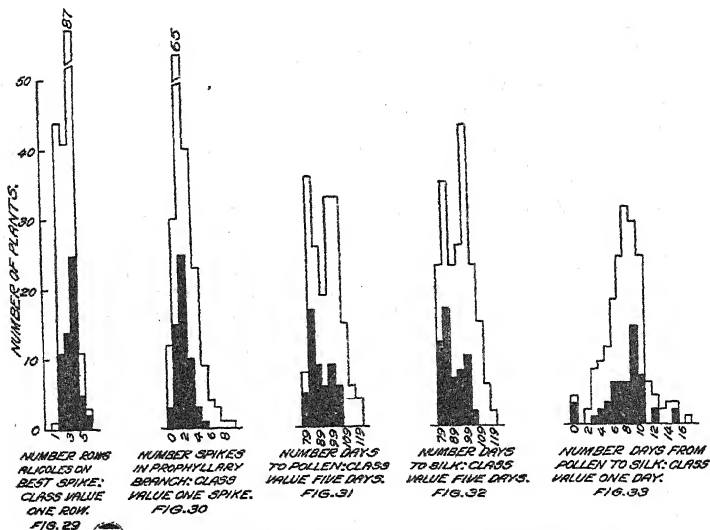
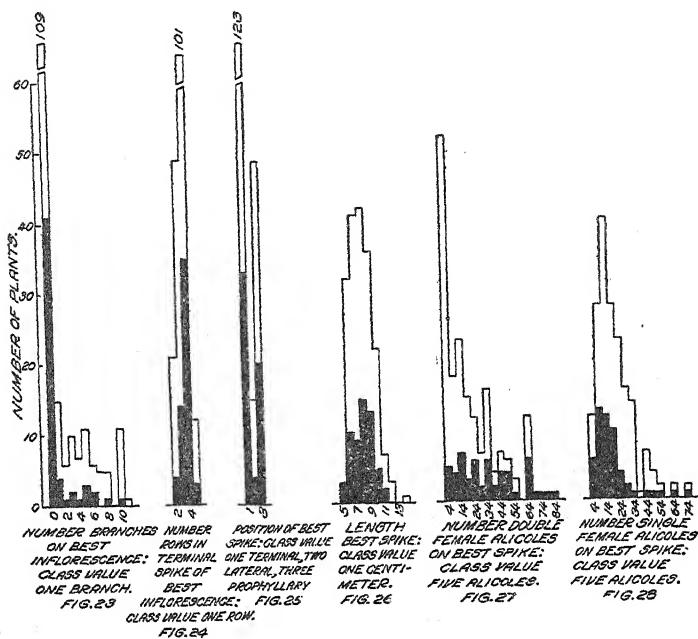
- FIG. 1.—Height; frequency distribution of plants in  $F_2$ . Class value, 1 decimeter. Shaded portion represents plants classed as crinkly.
- FIG. 2.—Height of tallest sucker; frequency distribution of plant in  $F_2$ . Class value, 1 decimeter. Shaded portion represents plants classed as crinkly.
- FIG. 3.—Total sucker height; frequency distribution of plants in  $F_2$ . Class value, 1 meter. Shaded portion represents plants classed as crinkly.
- FIG. 4.—Number of suckers; frequency distribution of plants in  $F_2$ . Class value, 1 sucker. Shaded portion represents plants classed as crinkly.
- FIG. 5.—Leaves above; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as crinkly.
- FIG. 6.—Total number of leaves; frequency distribution of plants in  $F_2$ . Class value one leaf. Shaded portion represents plants classed as crinkly.
- FIG. 7.—Length of branching space; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as crinkly.
- FIG. 8.—Length of central spike; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as crinkly.
- FIG. 9.—Length of longest tassel branch; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as crinkly.
- FIG. 10.—Length glume; frequency distribution of plants in  $F_2$ . Class value, 1 mm. Shaded portion represents plants classed as crinkly.
- FIG. 11.—Number of tassel branches; frequency distribution of plants in  $F_2$ . Class value, 10 branches. Shaded portion represents plants classed as crinkly.



FREQUENCY DISTRIBUTION OF PLANTS IN  $F_2$ .  
SHADED PORTION REPRESENTS PLANTS CLASSED AS CRINKLY.

## FIGURES 12 TO 22

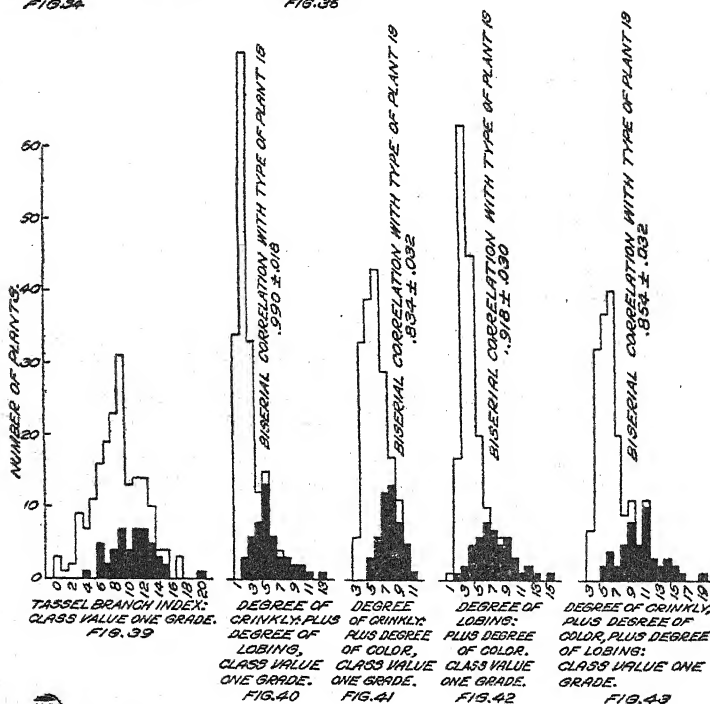
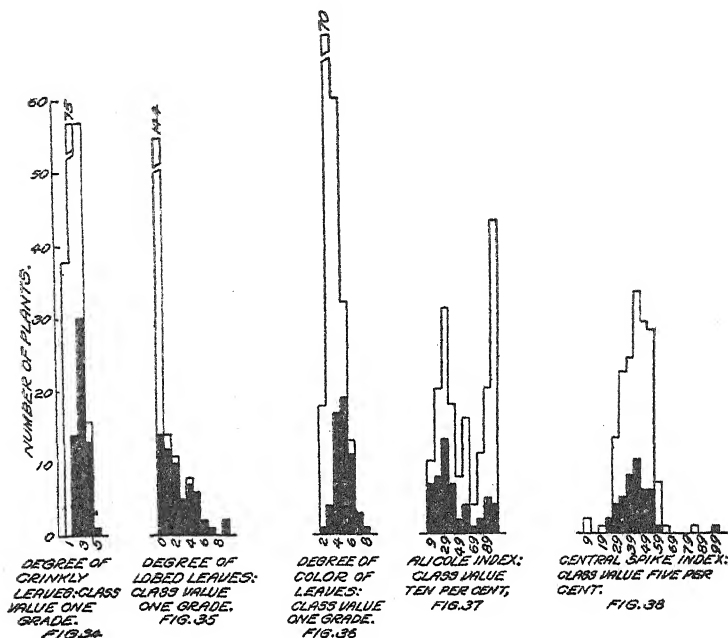
- FIG. 12.—Rows in central spike; frequency distribution of plants in  $F_2$ . Class value one row. Shaded portion represents plants classed as crinkly.
- FIG. 13.—Length of leaf; frequency distribution of plants in  $F_2$ . Class value, 1 decimeter. Shaded portion represents plants classed as crinkly.
- FIG. 14.—Width of leaf; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as crinkly.
- FIG. 15.—Width index; frequency distribution of plants in  $F_2$ . Class value, 1 per cent. Shaded portion represents plants classed as crinkly.
- FIG. 16.—Length of sheath; frequency distribution of plants in  $F_2$ . Class value, 1 centimeter. Shaded portion represents plants classed as crinkly.
- FIG. 17.—Position of longest leaf; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as crinkly.
- FIG. 18.—Leaves above best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as crinkly.
- FIG. 19.—Leaves on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as crinkly.
- FIG. 20.—Length of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as crinkly.
- FIG. 21.—Length of ear stalk of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 5 cm. Shaded portion represents plants classed as crinkly.
- FIG. 22.—Length of female portion of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as crinkly.



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FREQUENCY DISTRIBUTION OF PLANTS IN  $F_2$ .  
SHADED PORTION REPRESENTS PLANTS CLASSIFIED AS CRINKLY.

## FIGURES 23 TO 33

- FIG. 23.—Number of branches on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one branch. Shaded portion represents plants classed as crinkly.
- FIG. 24.—Number of rows in terminal spike of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as crinkly.
- FIG. 25.—Position of best spike; frequency distribution of plants in  $F_2$ . Class value, one terminal, two lateral, three prophyllary. Shaded portion represents plants classed as crinkly.
- FIG. 26.—Length of best spike; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as crinkly.
- FIG. 27.—Number of double female alicoles on best spike; frequency distribution of plants of  $F_2$ . Class value, five alicoles. Shaded portion represents plants classed as crinkly.
- FIG. 28.—Number of single female alicoles on best spike; frequency distribution of plants of  $F_2$ . Class value, five alicoles. Shaded portion represents plants classed as crinkly.
- FIG. 29.—Number rows of alicoles on best spike; frequency distribution of plants of  $F_2$ . Class value, one row. Shaded portion represents plants classed as crinkly.
- FIG. 30.—Number of spikes in prophyllary branch; frequency distribution of plants of  $F_2$ . Class value, one spike. Shaded portion represents plants classed as crinkly.
- FIG. 31.—Number of days to pollen; frequency distribution of plants of  $F_2$ . Class value, five days. Shaded portion represents plants classed as crinkly.
- FIG. 32.—Number of days to silk; frequency distribution of plants of  $F_2$ . Class value, five days. Shaded portion represents plants classed as crinkly.
- FIG. 33.—Number of days from pollen to silk; frequency distribution of plants of  $F_2$ . Class value, one day. Shaded portion represents plants classed as crinkly.



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FREQUENCY DISTRIBUTION OF PLANTS IN  $F_2$ .  
SHADED PORTION REPRESENTS PLANTS CLASSED AS CRINKLY.



## FIGURES 34 TO 43

- FIG. 34.—Degree of crinkly leaves; frequency distribution of plants of  $F_2$ . Class value, one grade. Shaded portion represents plants classed as crinkly.
- FIG. 35.—Degree of lobed leaves; frequency distribution of plants of  $F_2$ . Class value, one grade. Shaded portion represents plants classed as crinkly.
- FIG. 36.—Degree of color of leaves; frequency distribution of plants of  $F_2$ . Class value, one grade. Shaded portion represents plants classed as crinkly.
- FIG. 37.—Alicole index; frequency distribution of plants of  $F_2$ . Class value, 10 per cent. Shaded portion represents plants classed as crinkly.
- FIG. 38.—Central spike index; frequency distribution of plants of  $F_2$ . Class value, 5 per cent. Shaded portion represents plants classed as crinkly.
- FIG. 39.—Tassel branch index; frequency distribution of plants of  $F_2$ . Class value, one grade. Shaded portion represents plants classed as crinkly.
- FIG. 40.—Degree of crinkly plus degree of lobing; frequency distribution of plants in  $F_2$ . Class value, one grade. Biserial correlation with type of plant is  $0.990 \pm 0.018$ . Shaded portion represents plants classed as crinkly.
- FIG. 41.—Degree of crinkly plus degree of color; frequency distribution of plants in  $F_2$ . Class value, one grade. Biserial correlation with type of plant is  $0.834 \pm 0.032$ . Shaded portion represents plants classed as crinkly.
- FIG. 42.—Degree of lobing plus degree of color; frequency distribution of plants in  $F_2$ . Class value, one grade. Biserial correlation with type of plant is  $0.918 \pm 0.030$ . Shaded portion represents plants classed as crinkly.
- FIG. 43.—Degree of crinkly plus degree of color plus degree of lobing; distribution of plants in  $F_2$ . Class value, one grade. Biserial correlation with type of plant is  $0.854 \pm 0.032$ . Shaded portion represents plants classed as crinkly.

## INHERITANCE OF MEASURED CHARACTERS

Although plant height is one of the distinguishing characteristics of crinkly, none of the measurements of height in the second generation showed indications of Mendelian segregation, the distributions being similar to those of the same character in the Tom Thumb-Florida cross (5). The same is true also of the number of suckers, of which *Euchlaena* has a great many and crinkly none. It is of interest to note that the mean number of suckers in the crinkly hybrid was only about one-fourth the mean number produced in the Tom Thumb hybrid.

In the number of leaves above the uppermost lateral inflorescence both  $F_1$  and  $F_2$  differed from previous hybrids of maize and teosinte. The mean of  $3.22 \pm 0.06$  nodes above the uppermost lateral inflorescence on the  $F_1$  plants is much greater than is usually the case, and is of particular interest, in view of the rather definite tendency of the crinkly variation to produce an ear in the axil of the uppermost leaf.

In the second generation the mean number was only slightly increased, resembling other hybrids in that the mean of the second-generation plants with respect to this character closely approximates that of the  $F_1$ . In range some plants were produced with more nodes above the uppermost lateral inflorescence than would be found in the maize parent, while none returned completely to the teosinte parent in this character.

In the total number of leaves produced on the main stalk, the inheritance was as expected and the distribution is regular. This applies also to the characters of the staminate inflorescence, though it is worthy of note that the number of tassel branches is very large, both in the first and second generation. In fact, the number of tassel branches in the first generation of the crinkly hybrid was greatly in excess of the number found in the  $F_1$  of the ramose hybrid, to be discussed, the positions being reversed in the second generations of these hybrids.

The number of days from germination to flowering and the alicole index are the only characters of the 38 which show indication of Mendelian or discontinuous inheritance, the distribution of the plants for these characters (fig. 31, 32, and 37) being bimodal. The bimodality of the distributions for the number of days to flowering are of particular interest, since crosses between early and late varieties of maize have never shown any indication of bimodality in the  $F_2$ . The alicole index showed a bimodal distribution in the second generation of the Tom Thumb-teosinte hybrid also, but while the greatest number of plants are found with a high index in the crinkly hybrid, the reverse is true for the Tom Thumb hybrid. This behavior follows from that of the  $F_1$ , where it was found that in the crinkly hybrid the single female alicole form of teosinte was partially dominant to the double female alicole of maize. The general character of the spikes, however, resembled the  $F_1$  of teosinte  $\times$  Tom Thumb in that they were flat and broad, suggesting a secondary ear of maize much more nearly than a spike of teosinte.

The inheritance of the proterogynous habit of teosinte, as expressed by the number of days from pollen to silk, was very different in the crinkly hybrid from previous maize teosinte hybrids, in that though the  $F_1$  was proterandrous by a mean of 2.08 days the  $F_2$  was proterogynous by an almost equal time, i. e., 2.04 days. In other hybrids the decidedly proterogynous habit of teosinte has failed to reappear in the  $F_2$ , while the  $F_1$  usually is even more proterandrous than the maize parent.

The proterogynous habit is one which may be influenced greatly by photoperiodism or other environmental factors. Many varieties of maize from South America, indigenous near the equator, are so decidedly proterandrous when grown in the United States as to interfere seriously with the production of seed.

Euchlaena seems to be proterogynous, regardless of the flowering date, and even when hastened into flower by artificially shortening the day.

Even in maize, though the plant as a whole usually is proterandrous, the individual inflorescences seem to be inherently proterogynous. If conditions force pistillate flowers to develop in an inflorescence normally staminate, the resulting flowers always are proterogynous. When gynandrous inflorescences are normal to the strain, as in tassel seed, they also are always proterogynous, while in the andromonoecious dwarf variation the full development of the anthers occurs after the silks have appeared.

None of the characters of the crinkly variations showed discontinuous inheritance, though most of the crinkly characteristics were recovered in the  $F_2$  in an even more extreme form than was found in the parental progeny. Crinkly and teosintelike plants from the  $F_2$ , together with their inflorescences, are shown in Plates 2, 3, 4, and 5.

Notwithstanding the failure to obtain bimodal distributions of these characters, the frequency polygons in figures 1, 7, 8, 9, 11, 13, 14, 15, 16, 34, 35, 36, 37, and 39, all clearly show the high degree of development, in the plants empirically classed as crinkly, of the characters commonly associated in this variation.

These relationships are measured by the coefficients of biserial correlations presented in Tables IV and V.

#### CORRELATIONS BETWEEN CHARACTERS

This brings us to the consideration of the whole problem of character relationships. Two questions may be answered by the data at hand: First, to what extent the character complex comprising the crinkly variation tends to remain associated; second, to what extent all or any of these characters are associated with other maize characters not involved in the crinkly variation.

Difficulties are encountered at the start in the proper interpretation of the correlation coefficients, since by direct means it is not possible to distinguish purely physiological relationships from those which are genetic.

Recourse may be had to the method suggested by Collins of comparing the degree of correlation in the  $F_1$  with that found in the  $F_2$  (2). Correlations in the  $F_1$  are held to be due to physiological relationships, while any differences between  $F_1$  and  $F_2$  correlations may be ascribed to genetic causes. The coefficients of correlation between all the characters studied in this hybrid are given in Table II.





TABLE II.—Coefficients of correlation among the characters of the plants of the first and second generations of the hybrid between crinkly maize and Florida teosinte—Continued

Character.	Length ear stalk, best infl.		Length ♀ portion, best infl.		Branches on best infl.		Rows terminal spike, best infl.		Position of best spike.		Length of best spike.		Rows of alcohols on best spike.		Double ♀ alcohols on best spike.		Single ♀ alcohols on best spike.					
	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>				
Height.....	.365	.503	-.014	-.318	.248	.352	D	.050	D	-.408	D	-.075	D	-.435	D	-.037	D	-.435	D			
Height of tallest sucker.....	.499	.355	-.139	-.208	.272	.185	D	.109	.237	D	.186	.247	D	.056	.162	D	.123	.063	.114	.127		
Total sucker height.....	.660	.330	-.330	-.231	.307	.089	D	.045	.185	D	-.106	.233	D	.000	.179	D	.261	.233	.476	.071		
Number of suckers.....	.374	-.084	.241	-.045	.159	.070	D	.010	.145	D	-.046	.084	D	-.000	.022	D	.229	.010	.723	.010		
Leaves above.....	-.026	.063	.310	-.004	.041	-.089	D	.222	.071	D	.030	.077	D	.366	.047	D	.169	.089	D	.044	.010	
Total number of leaves.....	D	-.001	.544	.233	.092	.351	D	-.081	.173	D	.218	.093	D	-.085	.084	D	.191	.198	D	.033	.014	
Length of branching space.....	.275	.110	D	.122	.201	.171	D	.127	.134	D	.077	.077	D	.224	.017	D	.067	.077	D	.206	.043	
Length of central spike.....	.162	.320	D	.166	.089	.000	.230	D	.216	.106	D	.066	.100	D	.169	.032	D	.039	.105	D	.243	.071
Length of longest tassel branch.....	.313	.247	-.024	.303	.173	.237	D	.000	.077	D	.098	.077	D	.207	.158	D	.091	.095	D	.183	.006	
Length of glume.....	.126	.266	D	.043	.045	.055	.145	D	.151	.028	D	.049	.027	D	.028	.084	D	.082	.063	D	.091	.123
Number of glumes.....	.227	.165	-.096	.185	.418	-.025	D	.090	.063	D	-.141	.020	D	.157	.084	D	.020	.045	D	.069	.142	
Rows in central spike.....	-.069	.115	.115	.....	D	.076	.....	.214	.....	D	.050	.....	.277	.....	.032	.....	.131	.....	.131	.....	.131	.....
Length of leaf.....	.285	.313	-.066	.142	.269	.255	D	.000	.105	D	-.066	.203	D	.010	.110	D	.165	.272	D	.098	.300	
Width of leaf.....	-.120	.230	.118	.145	.026	.108	D	.240	.134	D	.125	.032	D	.137	.110	D	.269	.190	D	.033	.237	
Width of index.....	-.250	.338	.102	.170	.183	.200	D	.166	.055	D	-.054	.138	D	.079	.007	D	.287	.202	D	.049	.340	
Length of sheath.....	.246	-.068	-.022	.055	.205	.232	D	.020	.114	D	-.028	.321	D	.161	.063	D	.208	.224	D	.178	.272	
Position of longest leaf.....	D	.022	.089	-.069	.008	.128	.134	D	.082	.037	D	.017	.155	D	.046	.142	D	.014	.045	D	.075	.027
Leaves above best inflorescence.....	D	.174	.063	.094	.095	D	.182	.154	D	.063	.247	D	.017	.219	D	.197	.105	D	.030	.287		
Leaves on best inflorescence.....	D	.236	.084	.111	.183	.101	.045	D	.075	.077	D	.125	.239	D	.010	.285	D	.069	.347	D	.033	.212
Length of best inflorescence.....	D	.961	.973	-.350	.359	.492	.624	D	.017	.110	D	.080	.171	D	.112	.257	D	.165	.219	D	.099	.055
Length ear stalk, best inflorescence.....	.....	.....	-.465	.326	.528	.560	D	.205	.114	D	.241	.100	D	.109	.295	D	.301	.027	D	.435	.219	
Length ♀ portion, best inflorescence.....	.....	.....	.....	.....	.510	.348	D	.205	.149	D	.115	.239	D	.045	.228	D	.081	.315	D	.149	.438	
Number branches on.....	.....	.....	.510	.348	.....	.....	D	.100	.149	D	.014	.332	D	.601	.155	D	.355	.185	D	.094	.438	
Rows terminal spike, best inflorescence.....	D	.528	.560	.205	.114	.100	.149	D	.014	.324	D	.207	.134	D	.601	.155	D	.355	.185	D	.094	.438
Position of best spike.....	D	.080	.171	.205	.114	.100	.149	D	.014	.324	D	.207	.134	D	.601	.155	D	.355	.185	D	.094	.438
Position of best spike.....	D	.080	.171	.205	.114	.100	.149	D	.014	.324	D	.207	.134	D	.601	.155	D	.355	.185	D	.094	.438
Rows of alcohols on best spike.....	.112	.257	.169	.205	.207	.238	D	.601	.155	D	.032	.205	D	.141	.047	D	.242	.504	D	.512	.726	
Double ♀ alcohols on best spike.....	.165	.281	.301	.027	.081	.315	D	.355	.185	D	.215	.032	D	.137	.386	D	.207	.123	D	.207	.123	
Single ♀ alcohols on best spike.....	.033	.212	D	.099	.055	D	.010	.350	D	.094	.088	D	.026	.130	.055	D	.102	.334	D	.102	.334	
Spikes in prophyllary.....	.339	.182	-.366	.247	.192	.231	D	.133	.055	D	.130	.055	D	.102	.334	D	.102	.334	D	.102	.334	
Days to pollen.....	.048	.182	-.232	.168	.146	.323	D	.456	.032	D	.081	.233	D	.092	.137	D	.317	.084	D	.419	.304	
Days to silk.....	D	.086	.375	.085	.347	.212	.485	D	.207	.045	D	.111	.207	D	.315	.226	D	.161	.045	D	.297	.378
Days pollen to silk.....	D	.229	.170	.179	.156	.156	.485	D	.301	.050	D	.022	.309	D	.182	.182	D	.309	.182	D	.207	.378
Degree of crinkly.....	.205	.....	.188	.....	.145	.....	.088	.....	.301	.050	D	.022	.309	D	.182	.182	D	.309	.182	D	.207	.378
Degree of lobing.....	.138	.....	.181	.....	.170	.....	.224	.....	.224	.010	D	.010	.247	D	.118	.118	D	.245	.118	D	.245	.118
Degree of color.....	.075	.200	-.461	.114	.149	.442	D	.070	.228	D	.169	.014	D	.163	.050	D	.764	.915	D	.866	.862	
Alcile index.....	-.247	.....	.....	.....	-.257	.....	.110	.....	D	.097	D	.097	D	.099	D	.131	.442	D	.442	D	.204	.....

Character.	Number of spikes in prophyllary.		Alcicle index.		Days to pollen.		Days to silk.		Days pollen to silk.		Degree of crinkly.		Degree of lobing.		Degree of color.		Type of plant.	
	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>	F <sub>2</sub>	F <sub>1</sub>
Height.....	.286	.267	.755	.734	.155	.237	.200	.335	D	.731	.327	.....	.256	.....	.249	.....	.....	.....
Height of tallest sucker.....	.318	.279	.400	.071	.149	.300	.174	.369	D	.065	.330	.....	.239	.....	.140	.....	.....	.....
Total sucker height.....	.386	.193	.572	.127	.067	.145	.032	.176	D	.115	.158	.....	.204	.....	.073	.....	.....	.....
Number of suckers.....	.259	.055	.519	.006	D	.083	.292	.128	.359	D	.125	.257	D	.235	D	.014	.....	.....
Leaves above.....	.116	.100	.066	.045	.206	.138	.176	.032	D	.046	.142	D	.042	D	.061	D	.181	.....
Total number of leaves.....	.048	.453	.066	.045	.536	.573	.544	.607	D	.046	.142	D	.042	D	.217	D	.181	.....
Length of branching space.....	.068	.084	.259	.026	D	.052	.183	.043	.185	D	.313	.114	D	.188	D	.137	D	.435
Length of central spike.....	.010	.305	D	.134	.105	.206	.490	.166	.593	D	.172	.439	D	.157	D	.137	D	.435
Length of longest tassel branch.....	.267	.123	.283	.045	D	.068	.017	.066	.053	D	.225	.095	D	.447	D	.238	D	.711
Length of plume.....	.036	.142	.446	.077	D	.068	.003	D	.105	D	.059	.131	D	.068	D	.044	D	.369
Number of tassel branches.....	.037	.084	D	.068	.171	.135	.....	.094	.105	D	.308	.005	D	.039	D	.123	D	.331
Rows in central spike.....	.122	.....	.....	.....	.105	.....	.....	.....	.114	.....	.....	.....	.249	.....	.036	.....	.....	.....
Length of leaf.....	.177	.036	.093	.288	.302	.035	.....	.....	D	.032	.138	.....	.454	.....	.394	.....	.....	.....
Width of leaf.....	.094	.155	.335	.215	.355	.354	.....	.....	.151	.228	.445	.....	.534	.....	.512	.....	.....	.....
Width of index.....	.141	.134	.331	.198	.425	.294	.....	.....	.093	.248	.363	.....	.674	.....	.586	.....	.....	.....
Length of sheath.....	.075	.105	.013	.278	.207	.114	.....	.....	D	.138	.182	.....	.439	.....	.459	.....	.....	.....
Position of longest leaf.....	.....	.....	.....	.....	.....	.....	.....	.....	D	.123	.110	D	.173	.....	D	.114	.....	.....
Leaves above best inflorescence.....	.028	.095	.033	.032	.127	.217	.....	.....	D	.105	.089	.....	.078	.....	.196	.....	.....	.....
Leaves on best inflorescence.....	D	.014	.252	.022	.190	.168	.....	.....	D	.105	.089	.....	.078	.....	.196	.....	.....	.....
Length of best inflorescence.....	.344	.195	.047	.082	.018	.168	.....	.....	D	.085	.362	.....	.260	.....	.155	.....	.....	.....
Length of stalk, best inflorescence.....	.359	.182	.075	.082	.018	.168	.....	.....	D	.085	.362	.....	.260	.....	.155	.....	.....	.....
Length of portion, best inflorescence.....	.360	.247	.440	.114	.216	.168	.....	.....	D	.085	.362	.....	.260	.....	.155	.....	.....	.....
Number branches on best inflorescence.....	.124	.251	.049	.228	.456	.293	.....	.....	D	.217	.385	.....	.145	.....	.170	.....	.....	.....
Rows terminal spike, best inflorescence.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Position of best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Length of best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Length of best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Row of peduncles on best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Single ♀ alioles on best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Single ♀ alioles on best spike.....	D	.133	.055	.028	D	.081	.103	D	.047	.213	.011	.045	D	.035	D	.010	.....	.....
Spikes in prophyllary.....	D	.010	.069	.360	.105	.136	.364	.095	.390	D	.032	.748	.....	.272	.....	.479	.....	.....
Days to pollen.....	.116	.364	.031	.253	.....	.....	.....	.....	D	.032	.748	.....	.272	.....	.479	.....	.....	.....
Days to silk.....	.095	.390	D	.099	.948	.908	.....	.....	D	.032	.748	.....	.272	.....	.479	.....	.....	.....
Days pollen to silk.....	.157	.276	D	.114	.275	.400	D	.032	.748	.....	.272	.....	.479	.....	.485	.....	.....	.....
Degree of crinkly.....	.157	.276	D	.114	.275	.400	D	.032	.748	.....	.272	.....	.479	.....	.485	.....	.....	.....
Degree of lobing.....	.183	.203	.169	.101	.270	.270	.....	.....	D	.032	.748	.....	.272	.....	.485	.....	.....	.....
Degree of collar.....	.183	.203	.169	.101	.270	.270	.....	.....	D	.032	.748	.....	.272	.....	.485	.....	.....	.....
Alcicle index.....	.360	.105	.004	.004	.460	.460	.....	.....	D	.032	.748	.....	.272	.....	.485	.....	.....	.....
Type of plant.....	.327	.....	.448	.....	.473	.....	.....	.....	D	.032	.748	.....	.272	.....	.485	.....	.....	.....

Since the correlations are given for both the  $F_1$  and the  $F_2$  populations, it seems unnecessary to discuss them in detail. An examination of the coefficients in Table II will show that many of the characters of maize tend to remain associated in inheritance, and a comparison of the coefficients in this table with those given in Table X for the brachytic hybrid and with those given in Table III of the Tom Thumb hybrid show also that the associated complex of characters is not the same for these three hybrids. Thus, it is apparent that the combination of the large number of suckers of teosinte with the multiple-rowed condition of the pistillate inflorescence of maize can be obtained more readily in the hybrid with crinkly ( $r = -0.000$ ) than in that with brachytic, ( $r = -0.202$ ), though this condition could not have been anticipated from the characteristics of the maize parents. From the practical standpoint of establishing combinations of the desirable characters of both species, this difference in the degree of relationship among the various characters in the several hybrids is encouraging in that it affords a basis for predicting that even where pronounced correlations are encountered between characters which it is desirable to separate, further hybrids with other varieties of maize probably will reveal weaker associations between these characters. In addition to the many instances of coherence, a study of Table II will show many inconsistencies between the  $F_1$  and the  $F_2$ , as well as several disherences which can not be explained satisfactorily in the light of our present knowledge. These must await further investigations for proper interpretation.

TABLE III.—Coefficients of correlation between the characters commonly affected in the modification of normal maize plants to those of the crinkly type<sup>a</sup>

	Height.	Central spike.	Branching space.	Number of branches.	Length of longest branch.	Length of blade.	Width of blade.	Width index.	Length of sheath.	Degree crinkly. <sup>b</sup>	Degree lobing. <sup>b</sup>	Degree color. <sup>b</sup>	Generation.
Central spike.....	0.335 .119												$F_1^1$
Branching space..	.570 .145	0.195 .032											$F_1^1$
Number of branches.	.404 .089	.126 .323	0.542 .055										$F_1^1$
Length of longest branch.	.618 .055	.622 .063	.549 .114	0.361 .247									$F_1^1$
Length of blade...	.694 .441	.205 .134	.480 .257	.438 .073	0.530 .105								$F_1^1$
Width of blade...	.041 .123	.014 .440	.040 .030	D. 130 .245	.130 .171	0.257 .235							$F_1^1$
Width index.....	.434 .327	.127 .378	.300 .149	.172 .138	.417 .171	.746 .691	0.788 .860						$F_1^1$
Length of sheath.	.598 .128	.373 .187	.391 .173	.330 .164	.531 .119	.671 .530	.383 .342	0.646 .512					$F_1^1$
Degree of crinkly <sup>b</sup>	.177 .256	.131 .286	.073 .186	D. 608 .039	.207 .447	.290 .454	.445 .534	.502 .674	0.368 .430				$F_1^1$
Degree of lobing <sup>b</sup>	.249 .628	.002 .316	.137 .452	.123 .331	.238 .711	.394 .883	.512 .619	.586 .928	.430 .722	0.348 .480			$F_1^1$
Degree of color <sup>b</sup>											0.495 .872		$F_1^1$
Type of plant <sup>b,c</sup>												0.756	$F_2$

<sup>a</sup> The coefficients are shown for both  $F_1$  and  $F_2$ . Negative coefficients (indicated by a minus sign) apply to those cases in which a high value for one character is correlated with a low value for the other.  $F_1$  coefficients greater than 0.257 are in excess of three times the error.  $F_2$  coefficients greater than .150 are significant. Coefficients indicated by D represent disherences.

<sup>b</sup> These characters are not found in the  $F_1$ .

<sup>c</sup> The coefficients with type of plant are calculated by the formula for biserial correlation. Biserial coefficients greater than 0.186 are in excess of three times the error.

#### CORRELATIONS BETWEEN CRINKLY CHARACTERS

The characters which are involved primarily in the modification of normal plants to those of the crinkly type have been assembled in Table



III. In attempting to determine to what extent the crinkly complex of characters tends to remain in combination it is desirable to eliminate as far as possible the correlations expected on physiological grounds. Thus there are logical and experimental reasons for expecting physiological correlations of height with the five following characters given in Table III: Central spike, branching space, number of branches, length of longest branch, and length of blade. The nature of the crinkly teosinte cross is such as also to provide genetic causes of correlations of height with these characters, but with width of leaf genetic factors presumably operate in one direction while physiological factors operate in the other.

Thus, the crinkly variation has short stalks and wide leaves as compared with teosinte, and if there is a genetic correlation between these characters a negative correlation between height and width of leaves would be expected in the  $F_2$ . Further, since physiological factors normally tend to produce a positive correlation between height and width of leaves, the net effect of genetic and physiological factors operating in opposite directions would be to reduce the correlation essentially to zero. This is the observed result, though the coefficient is negative ( $r = -0.041$ ), indicating possibly that the genetic factors were somewhat more effective than the physiological factors in bringing about the observed relationship.

When the correlation between these two characters of the  $F_1$  plants is examined it also is found to be negative. While the correlation is not significant, its departure from the 0.2 or 0.3 usually obtained with these characters and the fact that the correlation with leaf index also is negative seem to require some explanation. It can be assumed that some peculiar environmental condition caused an increase in height and an actual and relative decrease in width of leaf such as obtain under extreme conditions where the plants become spindling; but such an hypothesis can not be accepted with favor when the exceptionally sturdy nature of the plants is taken into account.

The alternative is to attribute the results to genetic causes. Thus, if either the crinkly or the teosinte parent were heterozygous for a dominant factor which controlled a character complex of height, length, and width of blade, and the corollary height factors, length of central spike and branching space, then the  $F_1$  in effect would be a back cross of this nature and the observed  $F_1$  correlations would be obtained. While such a condition is not beyond the realm of possibility, it certainly seems singular that the complex of characters involved is so essentially that by which the recessive variation crinkly differs from the normal form.

It may be less unreasonable to assume that in hybrids such as this, where a general blending of the characters takes place, occasionally in certain plants one parental type or the other becomes partially dominant. There then would be  $F_1$  individuals in which all the maize characters would be raised somewhat above the mean, and others in which they would be below the mean. Such behavior would result in a population consisting largely of plants intermediate between the parents, but with the tails of the distribution made up of the parental character combinations, being those cases where the threshold of dominance had been crossed. The second generation of these several  $F_1$  plants need not necessarily behave very differently, and the  $F_1$  correlations would be provided for.

It is well known that the degree of dominance is extremely variable, but this variability formerly has been thought of in connection with individual characters. There is evidence from other sources that variations

in dominance are constitutional, affecting many or all of the characters. A striking instance is afforded by hybrids between maize and *Euchlaena perennis*. In these hybrids there is a much higher degree of dominance of all the teosinte characters than has been found in hybrids with *E. mexicana*.

Regarding the three characters graded for the degree of resemblance to the crinkly parent and their relations to the measured characters and to themselves, since in these cases there was no variation in the  $F_1$  plants, all being normal, there can be no  $F_1$  correlations.

Taking up the degree of crinkly which represents an attempt to measure the wrinkling of the leaf blade, this character is found in the  $F_2$  to be significantly correlated with eight of the eleven characters and with the type of plant as arbitrarily classed. These correlations, some of them large, are all in the direction of coherences.

The degree of lobing which evaluates the amount of lobing at the base of the leaf is found to be associated with ten other characters and with the type of plant, while the degree of color is correlated with eight characters and with plant type.

Although only a few of these correlations are large, they indicate that the crinkly complex of characters forms a correlated group suggesting loose linkage.

Since the plants were classed into crinkly and noncrinkly from their general appearance, it becomes of interest to discover to what extent the three characters, leaf lobing, crinkling, and color, determined the class in which the plants were placed. From an examination of Table III it is found that the degree of lobing, the least conspicuous of the three characters, has the closest correlation with the type of plant, but that this correlation is not as close as that of width index with type of plant, this index being a measured character. It would appear, therefore, that the relative width of the leaves was an important factor in the classification of the plants.

Since general characteristics were considered in classifying the plants into two groups, it may be urged with reason that it was a combination of the degree of crinkly, lobing and color that influenced judgment in classifying the plants. To test this, the grades of the three characters were combined in several ways and the correlations of these various combinations with plant class calculated. The coefficients are given with the frequency polygons in figures 40 to 43. It is found that the combination of degree of crinkly with degree of lobing is very closely correlated with plant class, the coefficient being  $r=0.99 \pm 0.03$ , the highest obtained with type of plant. While it is apparent that any plant having a high degree of crinkly and lobing would almost certainly be classed as crinkly, it is clear also that these two characters are not closely correlated;  $r=0.348$ . The regressions are  $0.181 \pm 0.029$  and  $0.669 \pm 0.109$  grades. Thus, for each change of one degree in the amount of crinkly there is a change of 0.18 degree in the amount of lobing, while for each change of degree of lobing there is a corresponding change of 0.67 degree in the amount of crinkly.

#### CORRELATION OF THE CRINKLY VARIATION WITH CHARACTERS OF MAIZE

Since all of the characters which are thought to be involved in the crinkly variations show significant correlations with type of plant, it seems reasonable to consider this arbitrary grouping as representing a definite segregation. The very high degree of correlation with leaf

index, however, is a cause for some misgiving, since it indicates a tendency on the part of the observer to class short, wide-leaved plants as crinkly. With this reservation in mind, the biserial correlations of the various characters with plant type may be examined. Arranging the characters in the order of their degree of correlation with type of plant, the ranking shown in Table IV is obtained.

TABLE IV.—*Biserial correlations of crinkly type of plant with the characters named a*

Rank.	Character.	Correlation.
1	Width index.....	0.928
2	Length of sheath.....	-.899
3	Length of leaf.....	-.883
4	Degree of lobing.....	.872
5	Degree of color.....	.756
6	Degree of crinkly.....	.722
7	Length of longest tassel branch.....	-.711
8	Height.....	-.628
9	Width of leaf.....	.619
10	Length of best inflorescence.....	-.497
11	Days to silk.....	-.495
12	Days to pollen.....	-.473
13	Height of tallest sucker.....	-.458
14	Length of branching space.....	-.452
15	Alicole index.....	-.448
16	Total number of leaves.....	-.445
17	Total sucker height.....	-.444
18	Number double ♀ alicoles.....	.442
19	Tassel branch index.....	.426
20	Rows in the central spike.....	.404
21	Number of suckers.....	-.334
22	Number of tassel branches.....	-.331
23	Number of spikes in prophyllary.....	-.327
24	Length of central spike.....	-.316
25	Length of glume.....	-.300
26	Leaves above best inflorescence.....	-.284
27	Number of branches on best inflorescence.....	-.257
28	Length of ear stalk of best inflorescence.....	-.247
29	Position of longest leaf.....	.224
30	Length ♀ portion of best inflorescence.....	.214
31	Number single ♀ alicoles.....	-.204
32	Leaves above uppermost branch.....	-.181
33	Number rows of alicoles on best spike.....	.131
34	Rows in terminal spike of best inflorescence.....	.110
35	Length of best spike.....	-.099
36	Position of best spike.....	.097
37	Leaves on best inflorescence.....	-.082
38	Central spike index.....	-.017
39	Days pollen to silk.....	.012

<sup>a</sup> Coefficients above .186 are in excess of three times the error. Correlations 26, 32, 35, 36, and 39 are disifferences.

The last 8 coefficients in this table may be disregarded, since they are less than three times their error. Of the others, 16 are characters which enter either directly or clearly indirectly into the classification of type of plant. Thus, height of plant doubtless was a factor which influenced classification, and for this reason such characters as total sucker height and number of suckers (both of which are correlated with height and in a sense simply are other measures of height) also are expected to be correlated with plant type. Eliminating such relationships as these, there are left 16 coefficients greater than three times their errors. A further reduction is possible with these 16, owing to the fact that in some instances

there are two or more measures of the same character. For instance, days to pollen and days to silk both obviously measure the same thing—season, while alicole index, number of double female alicoles and number of single female alicoles form another intercorrelated group, measuring essentially the same thing. With all such cases considered and reduced to a single coefficient, there remain 13 characters which are correlated directly with type of plant. These are given in italics in the last column of Table IV.

Of these 13 characters 12 are coherences showing a tendency for maize characters to be associated with the crinkly type of plant, while one is a disherence. This disherence is with leaves above the best inflorescence. Thus the crinkly plants have fewer nodes above the best lateral inflorescence than do the noncrinklies; a condition the reverse of that of the parental combination. In view of this fact it may be well to examine all the correlations with the character "leaves above the best inflorescence" shown in Table II.

In the second generation there are 36 possibilities for correlations with the character "leaves above best inflorescence," and 26 of these indicate disherences. Of the 36 coefficients, 17 are in excess of three times the error, but when the  $F_2$  correlations are considered and the physiological correlations eliminated, these 17 are reduced to 12. Of the 12 correlations the one dealing with leaves above the upper branch may be eliminated on morphological grounds, leaving 11, of which 10 are disherences. These disherent correlations are concerned with the following characters:

1. Height.
2. Rows in central spike.
3. Length of leaf.
4. Width of leaf.
5. Width index.
6. Position of longest leaf.
7. Leaves on best inflorescence.
8. Rows in terminal spike of best inflorescence.
9. Degree of crinkly.
10. Type of plant.

It may be of some significance that, of these 10, 6 are characters involved in the crinkly variation. It will be recalled that there is a tendency among crinkly plants to develop ears at the base of the tassel or in the axil of the upper leaf, and it seems not improbable that this tendency is manifested in the present hybrid. If this be true, it follows that the coefficients observed are coherences rather than disherences, since the character of few leaves above the upper branch becomes another measure of the crinkly variation. In the cross between Tom Thumb maize and Florida teosinte there were no significant disherences with the number of nodes above the uppermost inflorescence; a fact indicating that this character was associated with other characteristics of maize.

It may be concluded that "few leaves above the best inflorescence" in this case is a characteristic of the crinkly variation; it follows that all eight of the characters of maize not directly involved in the classification of the plants are correlated with the crinkly type.

It may be urged that while a character such as total leaves would not influence directly the classification of plants into two types, it might become correlated with type simply as a secondary relationship through its association with height. If this were true, the partial correlation of type with total leaves for constant height should be zero, when it actually is  $r = -0.433 \pm 0.059$ .

It seems clear that such characters as season, total leaves and the several measures of the inflorescences all are correlated with the variation known as crinkly, though none are characters by which the crinkly type is differentiated from the normal, and their correlation with type is not due to their association with some one crinkly characteristic.

Since the amount of leaf lobing was closely correlated with the crinkly type, and, furthermore, since this character would be expected to have little weight in the classification of plants, it may be well to examine the correlations of this character with all others. These correlations are given in Table V. With this character the usual correlation formula can be applied with but two exceptions—type of plant and alicole index. As in Table IV, the characters are arranged in the order of their degree of relationship with the lobing of the leaves. Thirteen of the 36 coefficients are less than three times their errors; of these, seven were significantly correlated with plant type, but not one of the seven is significantly correlated with the degree of leaf lobing.<sup>a</sup>

TABLE V.—*Product moment correlations of the degree of lobing with the characters named a*

Rank.	Character.	Correlation.
1	Type of plant. ....	<i>b</i> 0.872
2	Leaf index. ....	.674
3	Width of leaf. ....	.534
4	Degree of color. ....	.495
5	Length of leaf. ....	-.454
6	Length of longest tassel branch. ....	-.447
7	Length of sheath. ....	-.430
8	Degree of crinkly. ....	.348
9	Days to silk. ....	-.272
10	Days to pollen. ....	-.270
11	Length of best inflorescence. ....	-.269
12	Length of ear stalk of best inflorescence. ....	-.265
13	Height. ....	-.256
14	Rows in the central spike. ....	.249
15	Number double ♀ alicoles. ....	.245
16	Height of tallest sucker. ....	-.239
17	Number of suckers. ....	-.235
18	Total sucker height. ....	-.204
19	Number of spikes in prophyllary. ....	-.203
20	Length ♀ portion of best inflorescence. ....	.188
21	Length of branching space. ....	-.186
22	Alicole index. ....	<i>b</i> -.169
23	Length of central spike. ....	-.157
24	Number of branches on best inflorescence. ....	-.145
25	Number of rows alicoles on best spike. ....	.118
26	Leaves above best inflorescence. ....	-.103
27	Rows in terminal spike of best inflorescence. ....	.088
28	Total number of leaves. ....	-.086
29	Length of glume. ....	-.080
30	Length of best spike. ....	.076
31	Number of single ♀ alicoles. ....	-.042
32	Leaves above. ....	-.042
33	Number of tassel branches. ....	-.039
34	Position of best spike. ....	-.035
35	Days pollen to silk. ....	.024
36	Leaves on best inflorescence. ....	.010

<sup>a</sup> Coefficients in excess of 0.150 are greater than three times the error.

<sup>b</sup> Biserial correlations.

<sup>c</sup> There were eight coefficients with type of plant which were less than three times their error, but one of these, that with central spike index, does not appear in the correlation with the degree of lobing. It was omitted from all product moment correlations to facilitate the calculations by means of punched cards. The available cards were too short to accommodate all the characters.

The correlation between the rank of the characters in regard to correlation with type of plant and their rank in regard to correlation with degree of lobing is  $0.21 \pm 0.11$ , which is not a very close agreement. Not only are the characters not correlated with the degree of lobing in the same order as with type of plant, but the degree of relationship also is much weaker with lobing than with plant type. In general, however, characters of maize not involved in the crinkly variation are found correlated with the crinkly character lobing, clearly indicating genetic linkage of these numerous maize factors with the gene for crinkly.

#### RAMOSE $\times$ TEOSINTE

Ramose is a variation in which the simple pistillate inflorescence of maize has been replaced by a compound structure somewhat resembling the tassel. Practically all the seed is borne on branches, the central axis bearing seeds only at the apex. In addition to the branched condition of the ear, the number of branches on the staminate inflorescence has been increased at the expense of the central spike, the latter being reduced greatly in length. The branches of the tassel decrease regularly in length from the base to the tip, and there is little differentiation between branches and paired spikelets. This gradual decrease in the length of the branches gives the tassel a characteristic conical appearance easily distinguishable from the normal form.

The ramose variation, first described by Gernert, was found in a field of the Leaming variety (9), and has since been found in a strain of sweet corn grown by J. M. Mack of Fallbrook, Calif., (12). In crosses with normal maize this character behaves as a simple Mendelian recessive, and reappears essentially unaltered in the second generation. In the first hybrids studied the branched condition of the ear and tassel behaved as a single character, but in the strain of sweet corn grown by Mr. Mack plants were found having typical ramose tassels with normal unbranched ears, showing that the apparently single character was capable of subdivision or that separate modifying factors existed for each inflorescence.

The ramose ear and relatively unspecialized tassel, as compared with the normal maize inflorescences, represent a reversion to a primitive type in this one character, and its combination with the nearest wild relative of maize might be expected to shed additional light on the origin of the present single-spiked pistillate inflorescence of maize.

The hybrid with teosinte was made using ramose maize as the female parent. A few  $F_1$  plants were raised in the greenhouse at Chula Vista, Calif., and the second generation to be discussed is the progeny of one of these plants. The remainder of the  $F_1$  seed was planted in the field with the  $F_2$ , and resulted in 44 plants, all normal with respect to the form of inflorescence, which was similar to that of other  $F_1$  maize-teosinte hybrids.

One hundred and nineteen  $F_2$  plants were grown, of which 29 were classed as ramose, the percentage being  $24.4 \pm 2.7$ . One of these plants is shown in Plate 6, A, and the pistillate inflorescences are shown in Plate 7. The classification was made before the pistillate inflorescences were harvested, and therefore was based entirely on the characteristics of the tassel. In these hybrid plants almost every lateral branch terminates in a staminate panicle, and the ramose nature of the tassel is in evidence over the entire plant. The fact that these inflorescences are

developing over a relatively long period of time obviates some of the difficulties in classification arising from the effect on branching of fluctuating environmental factors.

#### CHARACTERS MEASURED

Several characters which were measured in the crinkly-teosinte hybrid were disregarded in the ramosa hybrid, since the two parents were not strikingly different with respect to them, and several new characters which serve to differentiate the parents were added. The added characters are: (a) Length of upper branch of tassel; (b) best spike, double male; (c) best spike, mixed male and female; and (d) number of branches on the best spike.

LENGTH OF UPPER BRANCH OF TASSEL.—In ramosa, the upper branch of the tassel often is but a centimeter or two long while in teosinte it usually exceeds 10 centimeters in length.

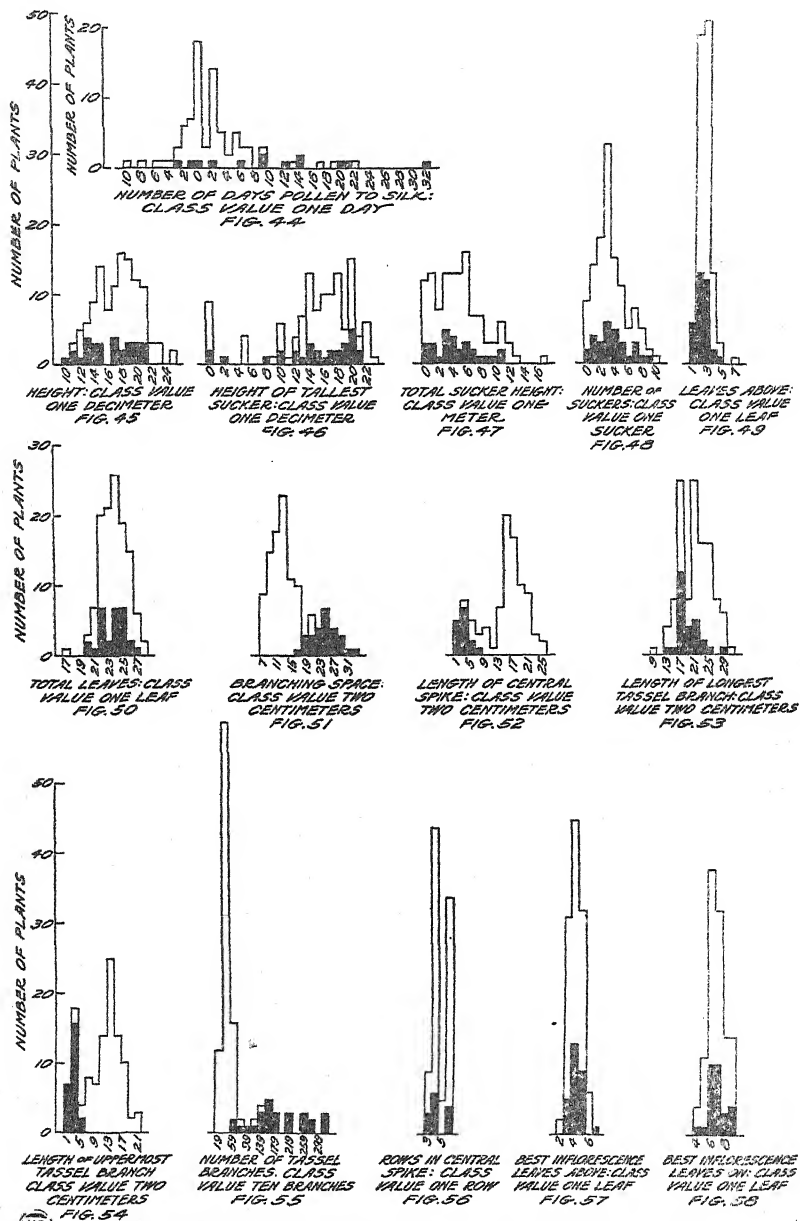
BEST SPIKE, DOUBLE MALE.—The number of alicoles having two staminate spikelets on the best spike. This type of inflorescence, though expected, was not found in the crinkly hybrid.

BEST SPIKE, MIXED MALE AND FEMALE.—The number of alicoles having one staminate and one pistillate spikelet on the best spike. Where double male alicoles are found, the spike usually has a few alicoles with spikelets of both sexes marking the transition from the pistillate to the staminate portion.

NUMBER OF BRANCHES ON THE BEST SPIKE.—This corresponds to the number of branches on the ears of ramosa plants, and is, of course, found only when the plant is of the ramosa type. This type of branching is not to be confused with the branched inflorescence of teosinte, where each branch is subtended by bracts. When the spike is but two-rowed, resembling the teosinte parent with respect to this character, and is combined with the branching typical of ramosa maize, the number of branches seldom exceeds 14, since they are limited to the number of alicoles. All the other characters used are identical with those of the crinkly-teosinte hybrid described on pages 540-543.

#### INHERITANCE OF MEASURED CHARACTERS

The biometrical constants for the plants of the  $F_1$  and  $F_2$  are given in Table VI, while the frequency polygons are shown in figures 44 to 75. In each of the figures the plants classed as ramosa are shaded.

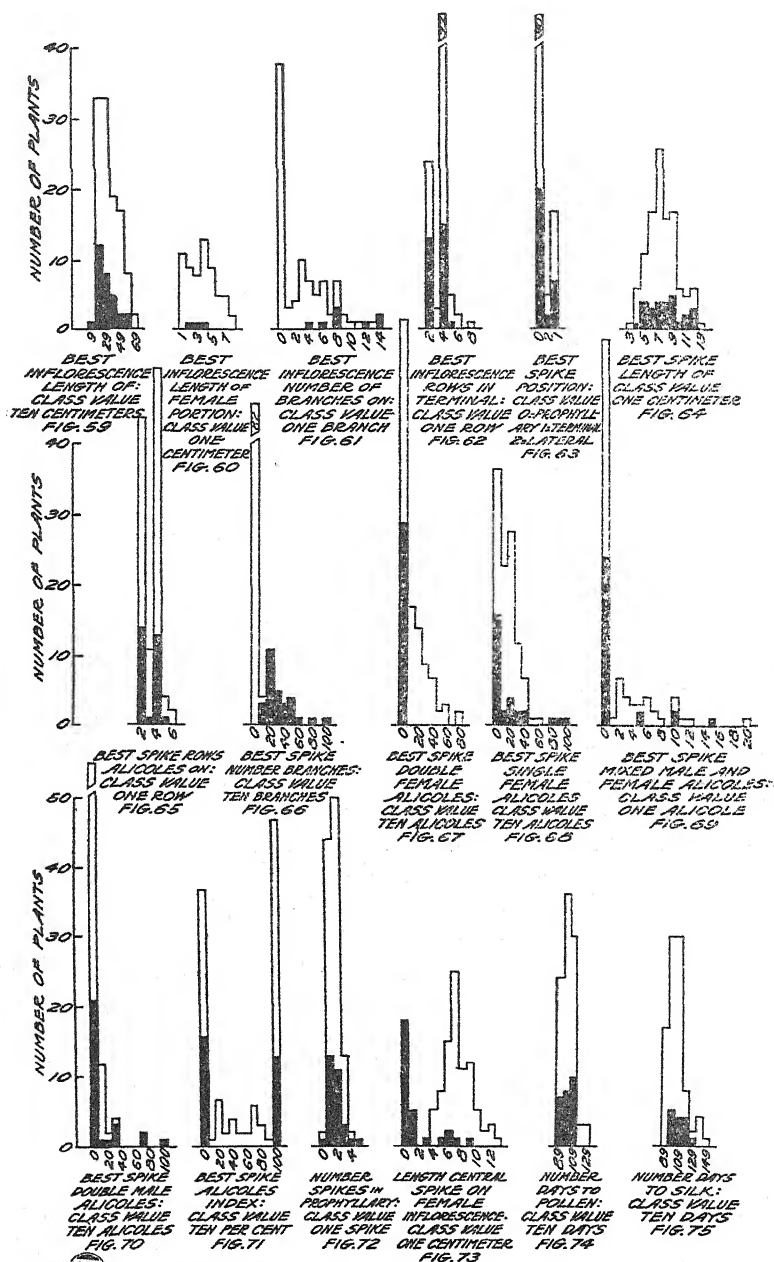


FREQUENCY DISTRIBUTION OF PLANTS IN F<sub>2</sub>  
SHADED PORTION REPRESENTS PLANTS CLASSED AS RAMOSE



## FIGURES 44 TO 58

- FIG. 44.—Number of days pollen to silk; frequency distribution of plants in  $F_2$ . Class value, one day. Shaded portion represents plants classed as ramose.
- FIG. 45.—Height; frequency distribution of plants in  $F_2$ . Class value, 1 decimeter. Shaded portion represents plants classed as ramose.
- FIG. 46.—Height of tallest sucker; frequency distribution of plants in  $F_2$ . Class value, 1 decimeter. Shaded portion represents plants classed as ramose.
- FIG. 47.—Total sucker height; frequency distribution of plants in  $F_2$ . Class value, 1 meter. Shaded portion represents plants classed as ramose.
- FIG. 48.—Number of suckers; frequency distribution of plants in  $F_2$ . Class value, one sucker. Shaded portion represents plants classed as ramose.
- FIG. 49.—Leaves above; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as ramose.
- FIG. 50.—Total leaves; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as ramose.
- FIG. 51.—Branching space; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as ramose.
- FIG. 52.—Length of central spike; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as ramose.
- FIG. 53.—Length of longest tassel branch; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as ramose.
- FIG. 54.—Length of uppermost tassel branch; frequency distribution of plants in  $F_2$ . Class value, 2 cm. Shaded portion represents plants classed as ramose.
- FIG. 55.—Number of tassel branches; frequency distribution of plants in  $F_2$ . Class value, 10 branches. Shaded portion represents plants classed as ramose.
- FIG. 56.—Rows in central spike; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as ramose.
- FIG. 57.—Leaves above best inflorescence frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as ramose.
- FIG. 58.—Leaves on best inflorescence frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as ramose.



## FIGURES 59 TO 75

FIG. 59.—Length of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 10 cm. Shaded portion represents plants classed as ramose.

FIG. 60.—Length of female portion of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as ramose.

FIG. 61.—Number of branches on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one branch. Shaded portion represents plants classed as ramose.

FIG. 62.—Rows in terminal spike of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as ramose.

FIG. 63.—Position of best spike; frequency distribution of plants in  $F_2$ . Class value, 0 for prophyllary, 1 for terminal, 2 for lateral. Shaded portion represents plants classed as ramose.

FIG. 64.—Length of best spike; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as ramose.

FIG. 65.—Rows of alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as ramose.

FIG. 66.—Number of branches on best spike; frequency distribution of plants in  $F_2$ . Class value, 10 branches. Shaded portion represents plants classed as ramose.

FIG. 67.—Double female alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, 10 alicoles. Shaded portion represents plants classed as ramose.

FIG. 68.—Single female alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, 10 alicoles. Shaded portion represents plants classed as ramose.

FIG. 69.—Mixed male and female alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, one alicole. Shaded portion represents plants classed as ramose.

FIG. 70.—Double male alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, 10 alicoles. Shaded portion represents plants classed as ramose.

FIG. 71.—Best spike, alicole index; frequency distribution of plants in  $F_2$ . Class value, 10 per cent. Shaded portion represents plants classed as ramose.

FIG. 72.—Number of spikes in prophyllary; frequency distribution of plants in  $F_2$ . Class value, one spike. Shaded portion represents plants classed as ramose.

FIG. 73.—Length of central spike on female inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as ramose.

FIG. 74.—Number of days to pollen; frequency distribution of plants in  $F_2$ . Class value, ten days. Shaded portion represents plants classed as ramose.

FIG. 75.—Number of days to silk; frequency distribution of plants in  $F_2$ . Class value, ten days. Shaded portion represents plants classed as ramose.

Height is not a feature of the ramosa variation, as it is of crinkly, and no bimodality in the various measures of this character was to be expected. The figures will facilitate a comparison of the distributions of the plants, with respect to these characters in this hybrid, with the distributions of the crinkly and brachytic hybrid.

A comparison of the distribution of the first six characters in the crinkly (fig. 1 to 6) and ramosa hybrids (fig. 45 to 50) shows a striking similarity, and emphasizes the failure of the semidwarf stature of crinkly to behave as a simple character.

As in the case of the hybrid with crinkly, many  $F_2$  plants of the ramosa hybrid were proterogynous, though the mean number of days from pollen to silk for the whole population and for each of the two groups comprising it was a positive value.

Bimodality is evident in the distributions for the several characters indicating the type of staminate inflorescence (fig. 51 to 55), and a very high correlation with the ramosa type also is apparent. It must not be overlooked, however, that in each case there are intermediate plants.

The frequency distributions that measure the branching of the pistillate inflorescence (fig. 61 and 66) also are bimodal, as well as the alicole index (fig. 71), which in this hybrid presents the best evidence as yet obtained for the discontinuous inheritance of this character. The  $F_1$  plants have a high percentage of single female alicoles, showing a partial dominance of this teosinte characteristic over the maize parent, and bimodality of the  $F_2$  is therefore not unexpected. The majority of the plants would be expected to have a high percentage of single female alicoles, which proves to be the case; and in this respect the ramosa hybrid resembles the crinkly hybrid and, like it, differs from the hybrid with Tom Thumb (5).

It will be observed (fig. 67) that none of the plants classed as ramosa had double female alicoles. This condition may be related to the almost complete sterility of these plants, especially as they approach teosinte in the character of the number of rows of alicoles. Of the very few seeds borne by these ramosa plants, all were on inflorescences that approximated maize in the number of rows of alicoles, and none was borne by the plants which resembled teosinte. Several plants with branched pistillate inflorescences were found that had but two rows of alicoles; but these plants were so completely sterile that there was no evidence of even the formation of spikelets; and the feathery branches, though not at all succulent, suggest the sterile "cauliflower" inflorescence of the pod-ramosa hybrid (3). (See Pl. 7.) In none of these two-rowed inflorescences was there found more than one branch from an alicole, though these inflorescences sometimes were ramified further.

The almost complete sterility of the ramosa segregates of the teosinte hybrid would account partially for the high percentage of plants with single female alicoles; and it is not without significance from the standpoint of the origin of the ear, suggesting as it does that teosinte is even further specialized with respect to branched inflorescences than is maize.

This sterility indicates that the change of normal maize to the ramosa condition is not in the nature of a reversion to teosinte. While it is possible to obtain a complete series of fertile forms connecting the pistillate teosinte inflorescence with the inflorescence of maize, and a similar series connecting the normal maize inflorescence with that of the ramosa type, it is not possible so to connect the ramosa inflorescence with that

of teosinte. The rudiments of intermediate structures are found, but they remain abortive and sterile.

It is a moot question whether the simple pistillate inflorescence of maize represents the loss of a more specialized branched condition or has come about through a still further specialization of a branched inflorescence in which the branches have become united. The ramosa variation is the principal evidence in support of the former view, and it is clear that the branched condition of ramosa maize had nothing in common with the branched inflorescence of teosinte. The incompatibility of the ramosa form of branched inflorescence in combination with the other characters of the pistillate spike of teosinte seems all the more remarkable in view of the general tendency toward branching manifested throughout the teosinte plant, and including both vegetative and floral organs.

Although it might have been expected that the ramosa variation would be correlated with maize characters and that few nonramosa inflorescences with many rows of alicoles would be obtained, the contrary proved to be the case. Thus, while the number of  $F_2$  individuals grown from the ramosa hybrid was less than that of any of the other hybrids, the parental types of inflorescences characterizing the normal forms of the parent species were recovered more nearly than in other hybrids. The best approximation to a normal unbranched ear was found in the  $F_2$  of the ramosa hybrid, and an inflorescence typical of Durango teosinte also was obtained, though no spikes were found in either the crinkly or ramosa hybrids having seeds shaped like those of Florida teosinte (4). (See Pl. 7, D.)

Not only were maize-like unbranched pistillate inflorescences obtained, but even in the relatively small group of ramosa plants branched ears were found closely approximating the ramosa ears of maize. In none of the cases of maize-like ears, however, were the inflorescences as large as the normal ears of the variety from which the ramosa parent was obtained, the longest ear being less than 15 cm. in length.

The group of ramosa pistillate inflorescences embraced no other ancestral characters. Thus, as in maize, when fertile pistillate spikelets were produced, these always had the hardened outer glumes and reduced bract-like inner glumes characteristic of the spikelets of a maize ear. There was a tendency for the inflorescences to be staminate, but this largely is a measure of sterility, which was so pronounced in the ramosa plants. Among the ramosa inflorescences that approached the two-rowed teosinte spike, the specialized rachis, characteristic of the pistillate inflorescence of teosinte, is entirely lacking, the inflorescence resembling completely a branch of the staminate panicle, with the distance between the articulations greater than is the case in normal pistillate inflorescences.

#### CORRELATION OF THE RAMOSA VARIATION WITH CHARACTERS THAT DISTINGUISH MAIZE FROM TEOSINTE

When the biserial correlations with type of plant are examined there are found to be only eight coefficients exceeding three times their error. These coefficients are given in Table VII. The biserial method could not be applied to the characters that measured the degree of ramoseness, since the distribution of plants with respect to these characters was bimodal. Of the eight coefficients, five at first sight appear to be dis-herences, while only three, days pollen to silk, height, and length of

best inflorescence are coherences. Of the five coefficients which would seem to be disherences three are capable of another interpretation, the correlations with length of pistillate portion of best inflorescence, number of double staminate alicoles on best spike, and number of rows in the terminal spike of the best inflorescence. The almost complete sterility of the ramose plants acts in such a manner as to reduce the length of the pistillate portion of the best inflorescence, causing a negative correlation of ramose type with this character. The positive correlation with the number of double male alicoles on best spike also is a disherence as viewed from the standpoint of normal maize, but, again, the sterility of the pistillate inflorescence seems the true explanation. The failure to develop pistillate flowers was manifested in many cases by the production of staminate spikelets, and in some cases entire inflorescences were staminate. Sterility also seems the best explanation of the negative correlation with the number of rows of alicoles on the terminal spike of the best inflorescence.

TABLE VII.—*Biserial correlations of ramose type with the characters named<sup>a</sup>*

Character.	Correlation.
Days pollen to silk.....	0.76
Length ♀ portion of best inflorescence.....	-.60
Number of double ♂ alicoles on best spike.....	.46
Rows in the central spike.....	.42
Rows in terminal spike of best inflorescence.....	-.41
Length of longest tassel branch.....	-.36
Height.....	-.30
Length of best inflorescence.....	-.28
Days to silk.....	.24
Position of best spike.....	.22
Alicole index.....	-.14
Height of tallest sucker.....	-.13
Number of single ♀ alicoles on best spike.....	.13
Number rows of alicoles on best spike.....	.12
Leaves above best inflorescence.....	.12
Length of best spike.....	.10
Number of spikes in prophyllary.....	-.09
Total number of leaves.....	-.09
Number of suckers.....	.08
Leaves on best inflorescence.....	.08
Leaves above upper branch.....	.06
Number of mixed male and female alicoles.....	-.05
Days to pollen.....	-.03
Total sucker height.....	.00
Number of double ♀ alicoles on best spike.....	.00

<sup>a</sup> Coefficients in excess of 0.24 are greater than three times the error.

Two outstanding disherences remain, for which there seems to be no logical interpretation. These are the negative correlation of ramose type with the number of rows in the central spike of the tassel, and the length of the longest tassel branch. A many-rowed central spike clearly is a maize character, and since it is part of the terminal staminate inflorescence which showed no obvious signs of sterility in the group of ramose plants, there is no morphological explanation of the rather high negative correlation. The same is true also of the negative correlation with the length of the longest tassel branch, for which no explanation can be offered.

The two negative coefficients with length of best inflorescence, and the positive correlations with days pollen to silk, are all coherences expected on the hypothesis that the ramose variation is correlated with maize characters. None of the other characters listed have significant coefficients, but it is of interest to compare this series of relationships with those shown for the crinkly variation in Tables IV and V.

The absence of correlation between the ramose type and the number of rows of alicoles on the best spike seems worthy of note. Thus it seems clear from this hybrid that the ramose parent introduced the necessary factors for the production of normal unbranched ears quite independently of the branched character of the inflorescence, and, as a consequence, unbranched inflorescences were obtained closely resembling normal maize ears.

#### BRACHYTIC $\times$ TEOSINTE

Brachytic is a dwarf variation of maize in which the length but not the number of internodes is reduced, and all other organs remain full-sized. In crosses with plants of normal stature this type of dwarf behaves as a simple Mendelian character recessive to the normal form, reappearing in approximately 25 per cent of the  $F_2$  plants, apparently unaltered as to stature (11).

Only one  $F_1$  plant was obtained of the hybrid with teosinte, and from this self-pollinated plant a second generation of 290 plants was grown. Of these plants only 35, or  $12.1 \pm 1.3$  per cent were classed as brachytic, a departure from the expected 25 per cent, and too significant to be ascribed to chance. One of these plants is shown in Plate 6. While in many maize crosses the percentage of brachytic plants recovered in the  $F_2$ , like that in the case of most recessive characters, is below the expected, the departures usually are within four times the error. The percentage obtained in the teosinte hybrid is not referable readily to the interaction of two independent factors, since it lies midway between the 6.25 per cent expected where the character only appears when two recessive factors are homozygous, and the 18.75 per cent expected in cases where the character appears on the interaction of a dominant and a recessive factor.

Not only is the number of brachytic plants too low for a simple recessive character, but the distinction between normal and brachytic plants is not as clear as in crosses with normal maize. This difference between the maize and the teosinte hybrids is shown by a comparison of the graphs in figures 76 and 83. The failure to obtain definite segregation probably accounts in a large measure for the low number of brachytic plants, and perhaps is due to the introduction of modifying factors through the teosinte parent.

Two plants were found that had brachytic internodes on the lower half and normal internodes above, and one plant was found with a brachytic main stalk and normal suckers. Such instances are not unique nor confined to the hybrid with teosinte, similar plants having been found in maize hybrids after repeated back crossing of brachytic with normal plants.

All varieties of maize exhibit what may be construed as an indication of brachysm, and it is possible to view the origin of the brachytic variation as an example of homeosis, or the transfer of the shortened internodes of the ear stalk to those of the main culm.

In teosinte there is no such indication of brachysm, all branches having elongated internodes. The first indication of the contamination of teosinte with maize is in the direction of brachysm, as instanced by a shortening of the pistillate rachis, resulting in displacing the alignment of the seeds and a shortening also of the lateral vegetative branches.

The absence in teosinte of the hereditary factors that control the shortening of the internodes and their homologues in the lateral inflorescences of maize may be responsible for the failure of simple segregation in the  $F_2$ .

#### CHARACTERS MEASURED

Several characters not recorded in the crinkly and ramose teosinte hybrids were studied in the brachytic hybrid, while other characters used in the former hybrids were not considered. The added characters are: (a) Diameter of stalk; (b) average length of internodes on best inflorescence; (c) rank of pistillate inflorescence.

**DIAMETER OF STALK.**—This measurement is taken at the smallest place of the largest internode and is recorded in millimeters. In teosinte the average diameter of the stalk is about 2 cm., while in brachytic maize it averages about 4 cm.

**AVERAGE LENGTH OF INTERNODES ON BEST INFLORESCENCE.**—This measurement is designed to determine whether the brachytic nature of suckers is retained on the lateral branches borne above the ground. The length of the branch in centimeters is divided by the number of internodes. The internodes of teosinte branches usually exceed 10 cm. in length, and in brachytic maize seldom exceed 1 cm.

#### INHERITANCE OF MEASURED CHARACTERS

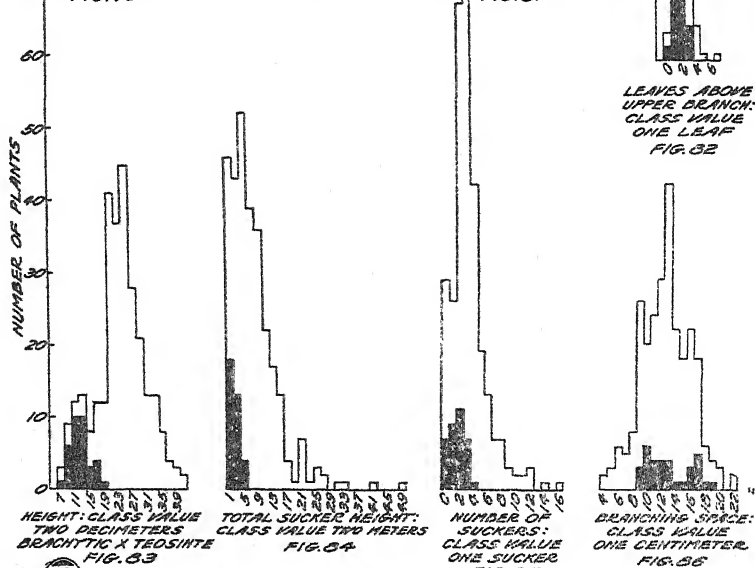
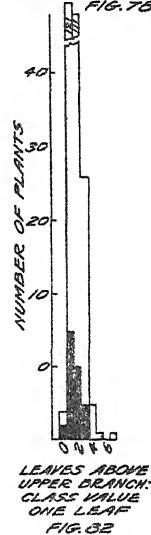
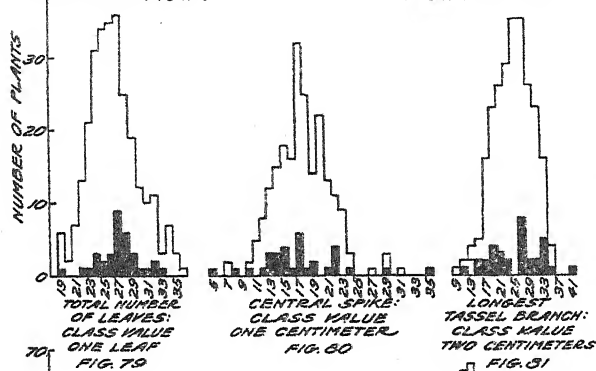
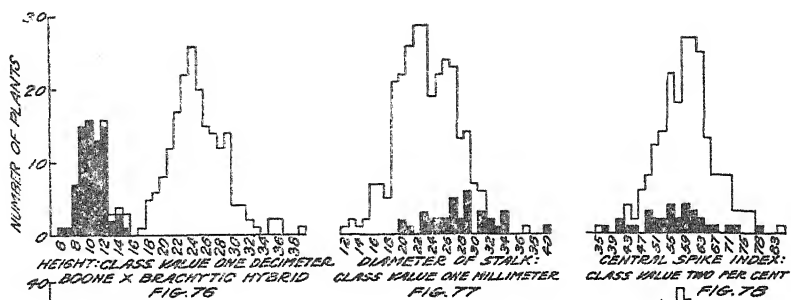
An examination of the frequency distribution shows that height of plant is the only characteristic having a bimodal distribution which can be attributed to the segregation of the brachytic character. Other bimodal characters are found, but these are not the result of the segregation of brachysm. Unlike the case of the crinkly and ramose hybrids, and similar to that of the Tom Thumb hybrid, no proterogynous plants were obtained in the  $F_2$ . The mean number of days from pollen to silk was  $5.52 \pm 0.13$ , which is very much less than the  $18.3 \pm 0.59$  observed in the Tom Thumb hybrid.

**RANK OF PISTILLATE INFLORESCENCE.**—The best pistillate spike from each plant was arranged in a series grading from teosinte to maize. This series was continuous, but for facility in handling the data an effort was made to combine the inflorescences into a few grades. Eight grades were established, grade one including only those spikes which were indistinguishable from pure teosinte, while grade eight embraced the most maize-like inflorescences. There were only four spikes of grade eight, and these resembled poorly developed maize ears. In the intermediate groups the inflorescences were classed as to the number of rows of spikelets, the amount of articulation of the rachis, etc. A fairly normal frequency distribution was obtained for these eight grades, which may be considered as indicating that this grouping of the inflorescences was a natural one. In all other maize-teosinte hybrids in which the Florida strain of teosinte has been used, the seeds of the most teosintelike inflorescences recovered in the  $F_2$  resembled the triangular seeds of the



Durango strain; none were obtained with the trapezoidal seeds typical of the Florida form. In the brachytic hybrid, however, several plants were found with seeds indistinguishable in form from those of the Florida teosinte, but much smaller in size and of very different color. In addition to recovering the seed shape, it should be noted that among the teosintelike inflorescences there are many having seeds which greatly exceed the seeds of teosinte in size, though exhibiting the shape characteristic of Durango teosinte. (See Plate 8.)

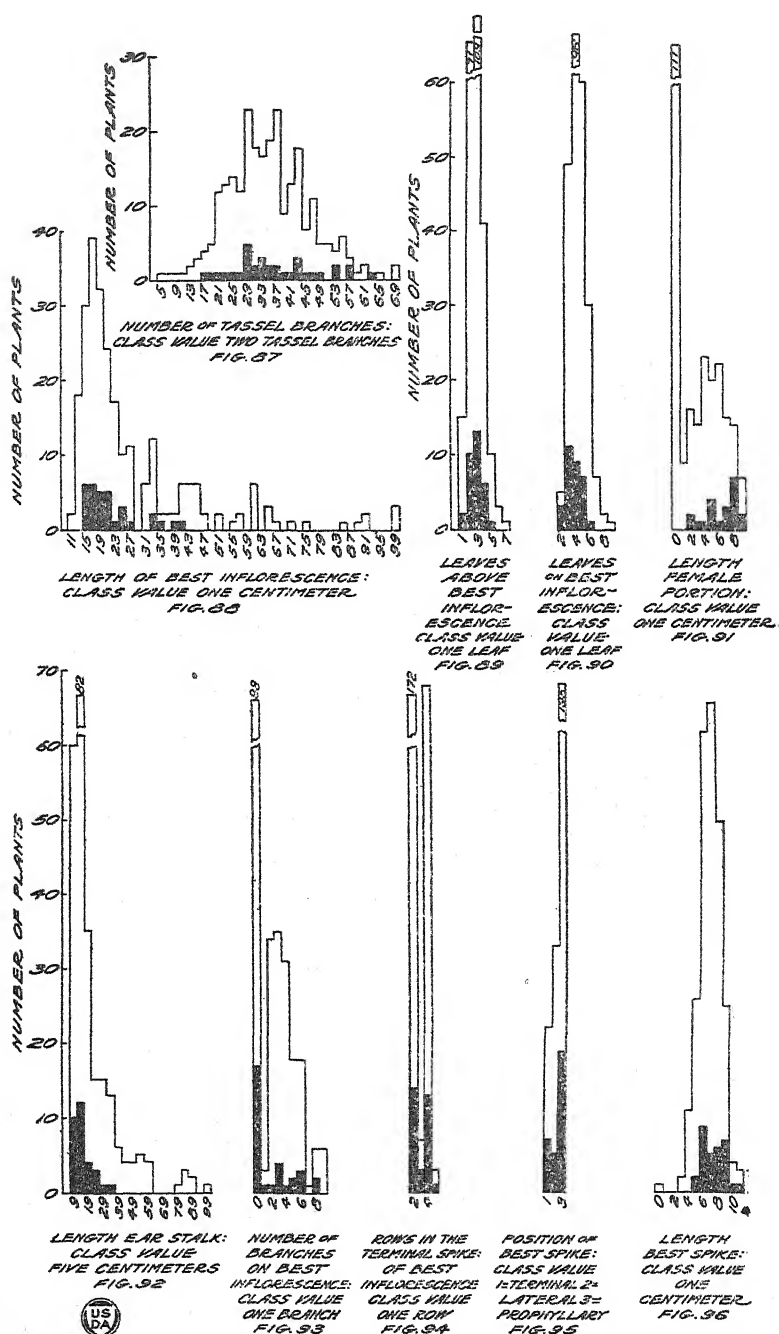
The brachytic variation has in its ancestry a pop corn with strongly beaked seeds, but the degree of beaking on the seeds of the brachytic strains has diminished until only rarely are the beaks perceptible. It was not without surprise, therefore, that inflorescences in the  $F_2$  of the brachytic-teosinte hybrid were obtained with a fantastic development of the beaked character, an example of which is shown in Plate 7, C.



FREQUENCY DISTRIBUTION OF PLANTS IN F<sub>2</sub>  
SHADED PORTION REPRESENTS PLANTS CLASSED AS BRACHYTIC

## FIGURES 76 TO 86

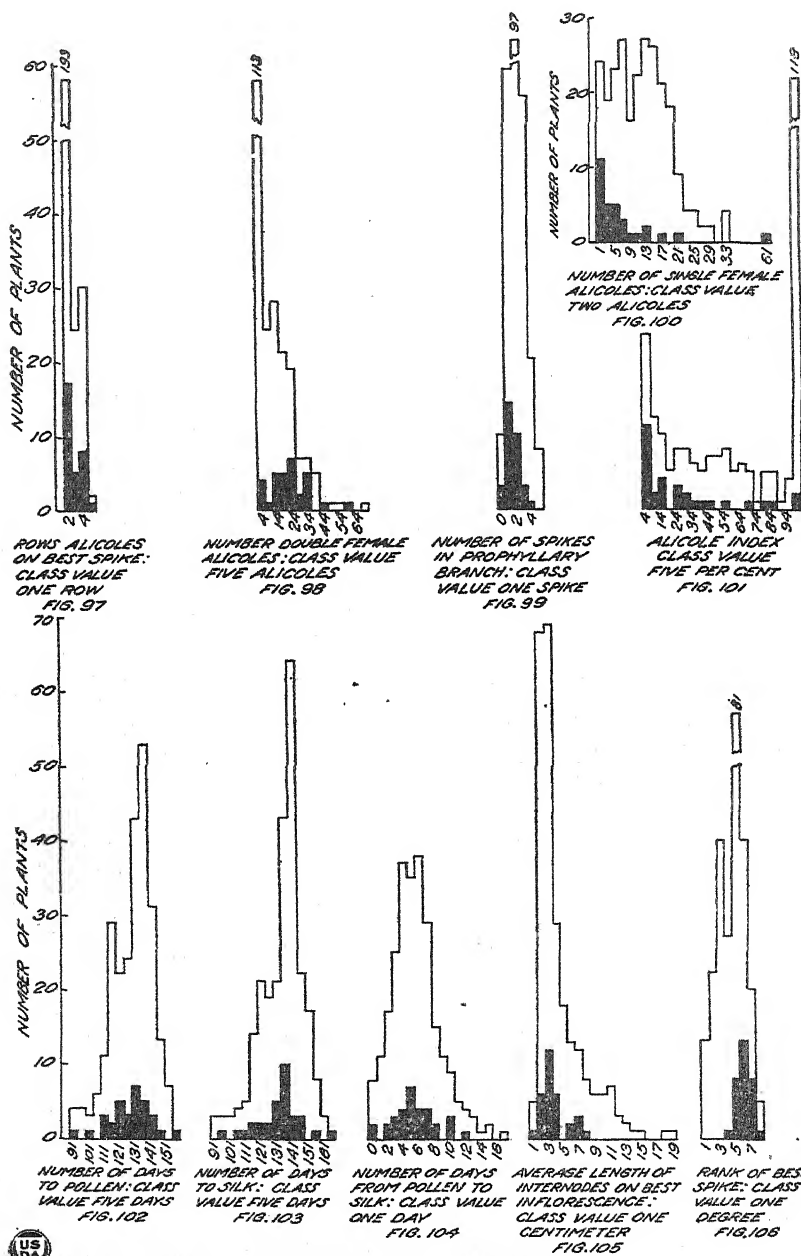
- FIG. 76.—Height; frequency distribution of plants in the  $F_2$  of a Boone  $\times$  brachytic hybrid. Class value, 1 decimeter. Shaded portion represents plants classed as brachytic.
- FIG. 77.—Diameter of stalk; frequency distribution of plants in  $F_2$ . Class value, 1 mm. Shaded portion represents plants classed as brachytic.
- FIG. 78.—Central spike index; frequency distribution of plants in  $F_2$ . Class value, 2 per cent. Shaded portion represents plants classed as brachytic.
- FIG. 79.—Total number of leaves; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as brachytic.
- FIG. 80.—Central spike; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.
- FIG. 81.—Longest tassel branch; frequency distribution of plants in  $F_2$ . Class value 2 cm. Shaded portion represents plants classed as brachytic.
- FIG. 82.—Leaves above upper branch; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as brachytic.
- FIG. 83.—Height; frequency distribution of plants in  $F_2$  of brachytic  $\times$  teosinte. Class value 2 decimeters. Shaded portion represents plants classed as brachytic.
- FIG. 84.—Total sucker height; frequency distribution of plants in  $F_2$ . Class value, 2 meters. Shaded portion represents plants classed as brachytic.
- FIG. 85.—Number of suckers; frequency distribution of plants in  $F_2$ . Class value, one sucker. Shaded portion represents plants classed as brachytic.
- FIG. 86.—Branching space; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.



FREQUENCY DISTRIBUTION OF PLANTS IN  $F_2$   
SHADED PORTION REPRESENTS PLANTS CLASSED AS BRACHYTIC

## FIGURES 87 TO 96

- FIG. 87.—Number of tassel branches; frequency distribution of plants in  $F_2$ . Class value, two tassel branches. Shaded portion represents plants classed as brachytic.
- FIG. 88.—Length of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.
- FIG. 89.—Leaves above best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as brachytic.
- FIG. 90.—Leaves on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one leaf. Shaded portion represents plants classed as brachytic.
- FIG. 91.—Length of female portion of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.
- FIG. 92.—Length of ear stalk of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 5 cm. Shaded portion represents plants classed as brachytic.
- FIG. 93.—Number of branches on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one branch. Shaded portion represents plants classed as brachytic.
- FIG. 94.—Rows in terminal spike of best inflorescence; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as brachytic.
- FIG. 95.—Position of best spike; frequency distribution of plants in  $F_2$ . Class value, 1 for terminal, 2 for lateral, 3 for prophyllary. Shaded portion represents plants classed as brachytic.
- FIG. 96.—Length of best spike; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.



FREQUENCY DISTRIBUTION OF PLANTS IN F<sub>2</sub>  
SHADED PORTION REPRESENTS PLANTS CLASSED AS BRACHYTIC

## FIGURES 97 TO 106

- FIG. 97.—Rows of alicoles on best spike; frequency distribution of plants in  $F_2$ . Class value, one row. Shaded portion represents plants classed as brachytic.
- FIG. 98.—Number of double female alicoles; frequency distribution of plants in  $F_2$ . Class value, five alicoles. Shaded portion represents plants classed as brachytic.
- FIG. 99.—Number of spikes in prophyllary branch; frequency distribution of plants in  $F_2$ . Class value, one spike. Shaded portion represents plants classed as brachytic.
- FIG. 100.—Number of single female alicoles; frequency distribution of plants in  $F_2$ . Class value, two alicoles. Shaded portion represents plants classed as brachytic.
- FIG. 101.—Alicole index; frequency distribution of plants in  $F_2$ . Class value, 5 per cent. Shaded portion represents plants classed as brachytic.
- FIG. 102.—Number of days to pollen; frequency distribution of plants in  $F_2$ . Class value, 5 days. Shaded portion represents plants classed as brachytic.
- FIG. 103.—Number of days to silk; frequency distribution of plants in  $F_2$ . Class value, 5 days. Shaded portion represents plants classed as brachytic.
- FIG. 104.—Number of days from pollen to silk; frequency distribution of plants in  $F_2$ . Class value, 1 day. Shaded portion represents plants classed as brachytic.
- FIG. 105.—Average length of internodes on best inflorescence; frequency distribution of plants in  $F_2$ . Class value, 1 cm. Shaded portion represents plants classed as brachytic.
- FIG. 106.—Rank of best spike; frequency distribution of plants in  $F_2$ . Class value, one degree. Shaded portion represents plants classed as brachytic.

The biometrical constants for the characters considered are given in Table VIII, and the frequency distributions are shown in figures 77-106. In each graph the shaded portion represents the plants classed as brachytic.

#### CORRELATION OF THE BRACHYTIC VARIATION WITH CHARACTERS OF MAIZE

When the biserial correlations with type of plant are examined it is found that there are 17 coefficients in excess of 3 times the error, while 13 are below this figure (Table IX). Of the 17, height and total sucker height should be eliminated, since these obviously are but other measures of the brachytic nature of the plants. Of the 15 remaining coefficients, 13 are coherences representing a tendency for the brachytic type of plant to reappear in the  $F_2$  accompanied by other maize characters. The two disifferences are the negative correlations of brachytic type with central spike index and with the number of leaves on the best inflorescence. Thus the brachytic plants, which usually have central spikes longer than those of normal maize plants and very much longer than those of teosinte plants, are found in this second generation to have relatively short central spikes. The coefficient, however, is only slightly in excess of 3.5 times the error, and may be due to chance. The negative correlation with the number of leaves on the best inflorescence seems incapable of a logical explanation, and must stand as a definite disifference.

TABLE VIII.—Biometrical constants for the second generation of the brachytic  $\times$  teosinte hybrid

Character.	Entire population.			Brachytic group.			Normal group.			Difference.			Fig. number of frequency polygon.
	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.	Amount.	P. E.	Amount-P. E.	
Height.....	22.75	6.94	0.278	11.74	2.71	0.309	24.29	5.89	0.353	12.55	0.399	31.48	83
Total sucker height..	7.04	6.50	.260	1.70	1.35	.154	7.77	6.62	.278	6.03	.318	18.95	84
Number of suckers....	3.26	2.49	.100	1.60	1.10	.125	3.49	2.54	.107	1.80	.165	11.21	85
Diameter of culm....	23.56	4.24	.170	27.91	4.33	.494	22.96	3.87	.162	-4.95	.520	9.52	77
Leaves above upper branch.....	1.73	.86	.034	1.56	.82	.098	1.75	.87	.037	.19	.105	1.81	82
Total number of leaves.....	26.44	3.36	.134	27.06	2.82	.327	26.35	3.44	.148	.71	.358	1.98	79
Central spike index....	58.61	7.82	.352	55.59	9.31	1.108	59.14	7.38	.369	3.55	1.167	3.04	78
Length of branching space.....	12.59	3.33	.140	13.03	2.99	.353	12.53	3.37	.152	.50	.384	1.30	86
Length of central spike.....	17.58	4.13	.186	17.09	5.68	.676	17.67	3.79	.190	.58	.701	.83	80
Number of tassel branches.....	34.27	11.25	.472	36.27	11.36	1.340	33.97	11.22	.505	-2.30	1.432	1.61	87
Length of longest tassel branch.....	24.15	5.34	.230	24.79	6.67	.787	24.05	5.07	.233	-.74	.820	.92	81
Leaves above best inflorescence.....	2.89	1.00	.043	2.81	.92	.109	2.90	1.02	.047	.09	.119	.75	89
Leaves on best inflorescence.....	4.38	1.15	.049	3.74	1.02	.123	4.47	1.15	.053	.73	.134	5.45	90
Length of best inflorescence.....	26.53	17.15	.737	21.34	7.08	.842	27.28	18.05	.830	5.94	1.182	5.02	88
Length of ear stalk of best inflorescence..	19.38	16.00	.688	13.23	5.96	.721	20.26	16.83	.774	7.03	1.058	6.64	92
Length of 2 portion of best inflorescence	2.75	2.94	.126	4.26	3.32	.402	2.53	2.82	.130	-1.73	.423	4.09	91
Number of branches on best inflorescence	2.43	2.44	.105	2.03	2.63	.318	2.48	2.42	.111	.45	.336	1.34	93



TABLE VIII.—*Biometrical constants for the second generation of the brachytic × teosinte hybrid—Continued*

Character.	Entire population.			Brachytic group.			Normal group.			Difference.			Fig. number of frequency polygon.
	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.	Mean.	$\sigma$	P. E.	Amount.	P. E.	Amount.	
Rows in terminal spike of best inflorescence.....	2.61	0.92	0.040	3.03	1.00	0.121	2.55	0.90	0.041	-0.48	0.128	3.75	94
Position of best spike.....	2.69	.62	.027	2.39	.83	.100	2.74	.55	.025	.35	.103	3.40	95
Length of best spike.....	6.85	1.51	.065	7.45	1.50	.182	6.76	1.52	.070	-.69	.195	3.54	96
Number rows of alicoles on best spike.....	2.36	.72	.031	2.77	.94	.114	2.30	.67	.031	-.47	.119	3.95	97
Number of double ♀ alicoles on best spike.....	8.76	11.78	.506	20.13	11.90	1.440	7.14	10.80	.497	-12.99	1.524	8.52	98
Number of single ♀ alicoles on best spike.....	10.99	7.78	.334	6.13	11.06	1.338	11.68	6.94	.319	5.55	1.375	4.04	100
Number of spikes in prophyllum.....	2.16	1.09	.047	1.52	.91	.110	2.25	1.10	.051	.73	1.106	.66	99
Alicole index.....	64.47	38.24	1.644	24.32	30.10	3.640	70.17	35.78	1.646	45.85	3.998	11.47	101
Days to pollen.....	126.85	12.96	.544	125.06	12.97	1.543	127.13	12.93	.595	2.07	.654	3.16	102
Days to silk.....	132.43	13.62	.572	130.50	13.58	1.616	132.71	13.58	.625	2.21	1.733	1.28	103
Days pollen to silk.....	5.52	3.01	.126	5.44	2.74	.326	5.53	3.05	.140	.09	.354	.25	104
Average length of internodes.....	4.40	3.07	.132	3.68	1.73	.209	4.50	3.20	.147	.820	.256	3.20	105
Rank of pistillate inflorescence.....	4.48	1.67	.072	6.00	.88	.106	4.26	1.63	.075	-1.74	1.634	1.07	106

TABLE IX.—*Biserial correlations of brachytic culms with the characters named a*

Character.	Correlation.
Height.....	-0.95 ± 0.03
Alicole index.....	-.63 ± .05
Diameter of stalk.....	.62 ± .05
Number of double ♀ alicoles.....	.58 ± .05
Rank of pistillate inflorescence.....	.56 ± .06
Total sucker height.....	-.55 ± .05
Number of suckers.....	-.40 ± .05
Number of single ♀ alicoles.....	-.37 ± .06
Number of spikes in prophyllary.....	-.35 ± .07
Rows of alicoles on best spike.....	.34 ± .07
Leaves on best inflorescence.....	-.34 ± .06
Length of ♀ portion of best spike.....	.31 ± .07
Position of best spike.....	-.29 ± .07
Rows in terminal spike of best inflorescence.....	.28 ± .07
Central spike index.....	-.25 ± .07
Length of best spike.....	.24 ± .07
Length of ear stalk best inflorescence.....	-.23 ± .07
Length of best inflorescence.....	-.19 ± .07
Average length of internodes on best inflorescence.....	.14 ± .07
Leaves above branch.....	-.12 ± .07
Total number of leaves.....	.11 ± .07
Number of tassel branches.....	.11 ± .07
Number of branches on best inflorescence.....	-.10 ± .07
Days to pollen.....	-.09 ± .07
Days to silk.....	-.09 ± .07
Branching space.....	.08 ± .07
Central spike.....	-.08 ± .07
Length of longest tassel branch.....	.07 ± .07
Leaves above best inflorescence.....	-.05 ± .07
Days pollen to silk.....	-.02 ± .07

a Correlations in excess of 0.22 are greater than three times the error.

CORRELATION AMONG THE CHARACTERS OF THE PLANTS OF THE  $F_2$ 

The coefficients of correlation among all the characters of the plants of the second generation are given in Table X. Of these, the most interesting are those of negative sign, since they represent the cases where large dimension of one character is associated with a small dimension in the other. In most such cases the interaction of physiological factors is eliminated, though there are several coefficients of negative sign which are purely mathematical in origin. Thus, the central spike index is of necessity negatively correlated with the length of the branching space, since the index represents the quotient obtained by dividing the length of the central spike by the sum of the length of the organ and the length of the branching space. There are others of a similar nature, but, considering the mass of coefficients in the table, they form a very small percentage.

HEIGHT AND TOTAL SUCKER HEIGHT.—There are three significant differences with height of plant, and two with total sucker height; but all of these can be discounted for physiological reasons. The non-linearity of the regression in the correlation involving height tends to reduce the coefficients, so that many of the coefficients not now in excess of three times the error probably are significant. It is worthy of note that with several characters, such as length of the pistillate portion of the best inflorescence and the number of double female alicoles, where physiological and genetic factors are operating in opposite directions, the genetic relationships are strong enough to bring about negative correlations.

TABLE X.—Coefficients of correlation among the characters of the plants of the second generation of the hybrid between brachytic maize and Florida teosinte<sup>a</sup>

Character.	Height.	Total sucker height.	Number of suckers.	Diameter of culm.	Leaves above.	Total number of leaves.	Central spike index.	Length of branching space.	Length of central spike.	Number of tassel branches.	Length of longest tassel branch.	Leaves above best inflorescence.	Leaves on best inflorescence.	Length of ear stalk of best inflorescence.
Height.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total sucker height.....	.678	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of suckers.....	.474	.915	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Diameter of culm.....	D .176	D .105	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Leaves above.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total number of leaves.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Central spike index.....	D .327	D .435	D .337	D .530	D .530	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of branching space.....	D .032	D .214	D .078	D .214	D .214	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of central spike.....	D .219	D .192	D .134	D .228	D .228	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of tassel branches.....	D .187	D .232	D .148	D .420	D .420	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of longest tassel branch.....	D .173	D .084	D .010	D .350	D .350	.....	.....	.....	.....	.....	.....	.....	.....	.....
Leaves above best inflorescence.....	D .095	D .045	D .014	D .045	D .045	.....	.....	.....	.....	.....	.....	.....	.....	.....
Leaves on best inflorescence.....	D .164	D .122	D .090	D .142	D .142	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of best inflorescence.....	D .337	D .272	D .142	D .243	D .243	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of ear stalk of best inflorescence.....	D .363	D .316	D .200	D .217	D .217	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of portion of best inflorescence.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of branches on best inflorescence.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rows in terminal spike of best inflorescence.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Position of best spike.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Length of best spike.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rows of alicotes on best spike.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of double ♀ alicotes on best spike.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of single ♀ alicotes on best spike.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Number of spikes in prophyllum.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Alicole index <sup>b</sup> .....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Days to pollen.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Days to silk.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Days pollen to silk.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Average length of internodes.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Rank of pistillate inflorescence.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

<sup>a</sup> Coefficients preceded by the letter "D" are those in which characters derived from different parents tend to remain associated. Coefficients in excess of 0.145 exceed three times their error.

<sup>b</sup> Bisceral correlations. Coefficients in excess of 0.184 are greater than three times their error.

TABLE X.—Coefficients of correlation among the characters of the plants of the second generation of the hybrid between brachytic maize and Florida teosinte—Continued

Character.	Length of $\varnothing$ portion of best inflorescence.	Number of branches on best inflorescence.	Rows of terminal spike of best inflorescence.	Position of best spike.	Length of best spike.	Rows of alioles on best spike.	Number of double alioles on best spike.	Number of single alioles on best spike.	Number of spikes in prophyllum.	Aliole index.	Days to pollen.	Days to silk.	Days pollen to silk.	Average length of internodes.	Rank of pistillate inflorescence.
Height.....	-.281	.232	-.182	.184	-.045	-.300	-.365	.214	.363	.349	.195	.185	D	.066	-.398
Total sucker height.....	-.314	.268	-.082	.195	-.045	-.100	-.337	.253	.283	.353	.155	.138	D	-.066	-.302
Number of suckers.....	-.284	.210	-.078	.107	-.078	-.262	-.343	.239	.267	.350	.126	.110	D	-.032	-.349
Diameter of culm.....	D	.295	.158	.319	.319	.107	.219	.020	.032	-.233	D	.138	D	-.060	.104
Leaves above.....	.219	-.176	.032	-.078	.138	.032	.184	-.100	.063	-.119	-.000	-.000	D	.063	.176
Total number of leaves.....	-.182	.277	.032	.078	.064	.032	.118	.100	.017	.086	.897	.554	D	.014	.219
Central spike index.....	.071	-.210	-.010	-.078	.110	D	D	.130	.032	D	.014	.022	D	-.032	D
Length of branching space.....	D	.340	D	.182	.237	.064	D	D	.064	.113	D	.118	D	.032	.114
Length of central spike.....	D	.340	D	.182	.237	.064	D	D	.064	.113	D	.118	D	.032	.114
Number of branches.....	D	.340	D	.182	.237	.064	D	D	.064	.113	D	.118	D	.032	.114
Number of longest tassel branch.....	D	.340	D	.182	.237	.064	D	D	.064	.113	D	.118	D	.032	.114
Leaves above best inflorescence.....	.078	.074	D	-.090	.095	.055	.095	.017	.045	-.022	.032	.045	D	-.071	D
Leaves on best inflorescence.....	D	.126	.055	.017	.010	.158	.118	.055	.110	D	.202	.152	D	.161	.045
Length of best inflorescence.....	.448	.570	-.179	.167	.045	-.045	.010	.063	.390	D	.105	D	D	.055	.861
Length of ear stalk of best inflorescence.....	-.466	.553	-.176	.167	.003	-.055	-.055	.078	.393	D	.090	D	D	.071	.908
Length of $\varnothing$ portion of best inflorescence.....	-.620	.431	.431	-.392	.173	.230	.331	.164	.235	-.264	.126	.148	D	.118	.296
Number of branches on best inflorescence.....	-.620	.431	.431	-.392	.173	.230	.331	.164	.235	-.264	.126	.148	D	.118	.296
Rows in terminal spike of best inflorescence.....	-.620	.431	.431	-.392	.173	.230	.331	.164	.235	-.264	.126	.148	D	.118	.296
Position of best spike.....	-.392	.366	.261	.263	.138	.425	.373	.165	.165	-.335	-.274	.277	D	.095	.219
Rows of alioles on best spike.....	.173	.044	D	.063	D	.364	.195	.090	.322	-.212	D	.084	D	.071	.197
Number of double $\varnothing$ alioles on best spike.....	.230	-.055	.425	.364	.138	.138	.460	.090	.107	-.200	-.182	.173	D	.044	.466
Number of single $\varnothing$ alioles on best spike.....	.311	.090	.371	.195	.311	.466	.598	.134	.105	.883	.351	.330	D	.010	.654
Number of spikes in prophyllum.....	-.235	.210	.105	.322	.105	D	.598	.105	.105	.660	.126	.110	D	.032	.256
Aliole index $\varnothing$ .....	.264	.055	.105	.322	.105	D	.598	.105	.105	.660	.126	.110	D	.032	.256
Days to pollen.....	.126	.071	-.274	D	.078	.182	.351	.126	.173	.345	.314	.974	D	.102	.395
Days to silk.....	D	.084	D	D	.185	.173	.314	.110	.197	.314	.974	.974	D	.102	.395
Days pollen to silk.....	.118	.090	D	.071	.014	D	D	.032	.152	.012	D	.385	D	.017	.381
Average length of internodes.....	.493	.568	.219	.197	.148	.090	.090	.063	.395	-.353	D	.014	D	.014	D
Rank of pistillate inflorescence.....	.296	-.078	.432	.197	.148	.066	.054	.256	.104	-.353	D	.014	D	.014	.142

<sup>b</sup> Biserial correlations. Coefficients in excess of 0.181 are greater than three times their error.

**NUMBER OF SUCKERS.**—While there are 15 significant correlations with number of suckers, all but four of these would be expected for both physiological and genetic reasons. Of the four which can be explained only on the basis of genetic relationship, the highest is with rank of the pistillate inflorescence, and all involve some measure of the form of this inflorescence. These correlations indicate clearly a tendency for plants with few suckers to bear maizelike inflorescences.

**DIAMETER OF CULM.**—Normal maize and teosinte differ pronouncedly in diameter of the culm, and this difference is greatly magnified when the maize parent is brachytic; yet, notwithstanding this fact, there are more disherences with diameter of culm in the brachytic-teosinte hybrid than with any of the other characters studied. It seems clear that physiological factors are able to mask most of the genetic relationships which exist, though the negative correlation with alicole index indicates that the most maizelike pistillate inflorescences will occur on plants with the thickest culms.

**LEAVES ABOVE UPPER BRANCH.**—With leaves above the upper branch, which is one of the best differentiating characters between maize and teosinte, there are no significant disherences. While the coefficients for none of the correlations with this character are high, with the exception of that with the leaves above the best inflorescence, the many negative coefficients where physiological and genetic factors are at work in opposite directions indicate the relative strength of the genetic relationships.

**TOTAL NUMBER OF LEAVES.**—With the character, "total number of leaves," there are two significant disherences, one of which can not be explained readily on the basis of the interaction of physiological factors; but in this case the coefficient of  $-0.148$  is but slightly in excess of three times the error, and with the number of coefficients under consideration several chance departures of this magnitude from complete independence are to be expected. Of the coherences, there are three clear examples of linkage, those with central spike index, length of the pistillate portion of the best spike, and rank of the pistillate inflorescence.

**CENTRAL SPIKE INDEX.**—Relatively long central spikes are an outstanding characteristic of brachytic maize, but from the standpoint of correlations with other characters the index is unsatisfactory. While there are only two significant disherences there is a complete absence of clear genetic linkage.

**BRANCHING SPACE.**—The length of the branching space also is unsatisfactory from the standpoint of correlations, since in many instances physiological and genetic factors operate in the same direction; and where disherences are found they obviously are due to the greater weight of the physiological factors.

**LENGTH OF CENTRAL SPIKE.**—This character has many of the objections of the other measures of the terminal panicle, though it should be observed that the negative correlations with days to pollen and silk, the former significant, show that linkage is involved with these complex characters.

**NUMBER OF TASSEL BRANCHES.**—There is one clear genetic relationship with this character, that with the length of the pistillate portion of the best spike, though, curiously enough, this relationship is not sustained with the other measures of the pistillate inflorescence.

**LENGTH OF THE LONGEST TASSEL BRANCH.**—While there are several significant disherences with this character, all can be ascribed to the interaction of physiological factors. There is one clear genetic correla-

LEAVES ABOVE THE BEST INFLORESCENCE.—With leaves above, where several genetic correlations were anticipated, none was found; and, while there are some unexpected disherences, these can be attributed to physiological causes.

NUMBER OF LEAVES ON THE BEST INFLORESCENCE.—With the number of leaves on the best inflorescence there are no significant correlations indicating disherences which can not be explained on physiological grounds; while the negative correlations with length of season seem clear examples of coherence brought about by genetic factors.

LENGTH OF THE BEST INFLORESCENCE.—With the length of the best inflorescence there are two clear genetic correlations, both negative; that with the rows in the terminal spike of the best inflorescence, and that with the length of the pistillate portion. The former is not large, but the latter is one of the best genetic correlations found. It seems clear that the plants with long branches tend to terminate in staminate rather than pistillate inflorescences, resembling teosinte in this respect.

LENGTH OF EAR STALK OF THE BEST INFLORESCENCE.—With length of ear stalk, which in teosinte is extremely long and in brachytic maize relatively short, essentially the same correlations are found as with the length of the best inflorescence, and, since these two measurements are closely correlated ( $r=0.952$ ), such behavior is a necessary consequence. The high correlation of 0.952 is not unexpected in view of the fact that by far the largest part of the total length of the best inflorescence is made up of the length of the ear stalk, the length of the flowering parts forming only a small part of the whole.

LENGTH OF THE PISTILLATE PORTION OF THE BEST INFLORESCENCE.—With the length of the pistillate portion of the terminal panicle of the best inflorescence there are fourteen coefficients of negative sign which exceed three time the error. These coefficients all indicate genetic correlations, and include such complex characters as height and season. In addition to the negative coefficients, there are several others of positive sign which also represent genetic rather than physiological relationship. It seems worthy of note that there are no significant disherences with this character.

NUMBER OF BRANCHES IN THE TERMINAL PANICLE OF THE BEST INFLORESCENCE.—With the number of branches on the terminal panicle of the best inflorescence there are two significant disherences, but, like so many of the preceding instances of this sort, both are capable of explanation on physiological grounds.

NUMBER OF ROWS IN THE TERMINAL SPIKE OF THE BEST INFLORESCENCE.—With the number of rows in the terminal spike of the best inflorescence there are no significant disherences; there are several rather high correlations with other characters of the flowering parts.

POSITION OF THE BEST SPIKE.—With the position of the best spike there is one outstanding disherence—that with days to pollen. In this case the plants having the best spikes in the axils of the prophylla, as in teosinte, are those with the shorter season. This negative correlation seems contrary to that expected, both genetically and physiologically. There is one outstanding correlation that clearly can be attributed to genetic causes, namely, that of  $-0.364$  with the rows of alicoles on the best spike.

LENGTH OF BEST SPIKE.—There are several significant genetic correlations with length of best spike, and no disherent correlations which can not be attributed to physiological factors.

**NUMBER OF ROWS OF ALICOLES ON BEST SPIKE.**—With the number of rows of alicoles on the best spike, which is one of the outstanding differences between maize and *Euchlaena*, there are no significant disherent correlations. It is interesting to observe that this character is negatively correlated with the measures of height as well as with season.

**NUMBER OF DOUBLE FEMALE ALICOLES ON BEST SPIKE.**—The number of double female alicoles and the number of single female alicoles, which in a certain sense are two complementary characters, both show significant genetic correlations, and no clear disherences. It seems noteworthy that where the number of double female alicoles is negatively correlated with the measures of height and season, the number of single female alicoles is positively correlated with these characters.

**NUMBER OF SPIKES IN PROPHYLLARY.**—The number of spikes in the prophyllary inflorescence has four significant correlations which represent disherences, but these all may be due to the interaction of physiological factors. On the other hand, there are several coefficients which can best be attributed to genetic causes.

**ALICOLE INDEX.**—This index measures the proportion of single female alicoles, which is one of the outstanding differences between maize and teosinte. It is of interest to observe that in this character there is but one significant disherence, and many clear genetic correlations.

**DAYS TO POLLEN AND DAYS TO SILK.**—With length of season as measured by the number of days from planting to anthesis and silking there are several significant disherences, the most outstanding being those with the number of spikes in the prophyllary inflorescence and the number of days between anthesis and the appearance of the first silk. The negative correlations with the number of spikes in the prophyllary inflorescence are contrary to expectation on both physiological and genetic grounds, while the positive correlations with days from pollen to silk might be attributed to the advancing season, with cool weather retarding the appearance of silking.

**DAYS POLLEN TO SILK.**—Of the 29 correlations with days from pollen to silk, there are only five in excess of three times the error; of these, two are disherences. The character as a whole does not seem to be a stable one, or one in which much confidence can be placed, though the teosinte parent always is proterogynous and the maize parent proterandrous.

**AVERAGE LENGTH OF INTERNODE.**—There is one significant disherence with the average length of the internode on the best branch, namely, that with diameter of culm; but since it has been apparent throughout that physiological factors have affected the diameter of the culm to the almost complete exclusion of genetic factors, no importance need be attached to this single disherence.

**RANK OF THE PISTILLATE INFLORESCENCE.**—The rank of the pistillate inflorescence, where the best spike of each plant was arranged in an ascending series from teosinte to maize, and this series was distributed among eight fairly definite groups, furnishes a composite measure evaluating all the characters of the spike. A fairly normal frequency distribution resulted from the grouping of these spikes, and the rank, therefore, may be taken as a fair estimate of the degree of resemblance of the spike to maize or teosinte. The correlations with rank should indicate to what extent the maizelike form of inflorescence is associated with the other characteristics of maize.

There are 21 significant correlations in a possible 29, and, without exception, these correlations indicate a tendency for the maizelike form of pistillate inflorescence to be associated in inheritance with other characters of maize.

The high correlation of rank with the number of double female alicoles (0.654), rather than with alicole index, arouses the suspicion that size was largely the determining factor in arranging the spikes. However, the correlation of rank with length of spike is only 0.148, and the partial correlation of rank with the number of double female alicoles for constant length of spikes is  $0.648 \pm 0.028$ . Mere size, therefore, could not have been an important factor in grading the ears.

Aside from the correlations of the several characteristics of the inflorescences with rank, the correlations with season seem worthy of note. Thus, it is apparent that the maizelike form of inflorescence is found on the earlier plants, a condition to be expected from the interaction of genetic factors.

#### SUMMARY

Several hybrids between normal maize and teosinte have been grown and, aside from variations in size, the first and second generations of such hybrids are very much alike. These generations are similar in inheritance to the hybrid between Tom Thumb pop corn and Florida teosinte, the inheritance of which has been reported previously.

In view of the seemingly complete blending of characters of both parents in such hybrids, it became of interest to analyze the behavior of some of the more strictly Mendelian variations of maize in hybrids with teosinte.

The present paper reports the inheritance of three striking Mendelian characters of maize, crinkly, ramose, and brachytic, in hybrids with the annual, teosinte, *Euchlaena mexicana*. As in hybrids with normal maize, all three variations are recessive in the hybrid with teosinte, and all three reappeared in the  $F_2$ , the two former in the expected monohybrid percentage of 25, but the last in only 12 per cent of the plants.

With the crinkly variation none of the measures of this character showed a bimodal distribution, though it was possible to classify the plants arbitrarily into two groups on their general appearance.

The several characteristics of the crinkly variation were found to be correlated among themselves, though in some cases the degree of correlation was low. The crinkly type of plant, as classed in the field, was found to be associated with maize characters not involved in the change from normal to crinkly, such as the form of the pistillate inflorescence, etc. This behavior indicates that there are genetic correlations among the maize characters in hybrids with teosinte, as well as a correlation between these characters and the mutant type, crinkly.

The classification of ramose plants in the  $F_2$  of the hybrid with teosinte was accomplished without difficulty and, while variation obviously existed among the plants classed as ramose, this was not as pronounced as in the ramose-Gordo hybrid (12). The variability was visible chiefly in the tassels, the pistillate inflorescences seeming to be more uniform. Thus, no intermediate pistillate spikes were found, all the ramose spikes being branched along the entire axis.

The teosintelike plants with ramose inflorescences, though apparently functioning normally with respect to the production of male gametes, were practically sterile with respect to the production of female gametes.



Not only were seeds not produced by these plants but spikelets were formed only rarely, the inflorescences usually consisting of naked feathery branches. This sterility, though most pronounced in the group more nearly approximating the teosinte parent with respect to other characters, was apparent also throughout the entire *ramose* group. Fertile seeds, however, were matured by the *ramose* plants that were most *maizelike* with respect to other characters.

Since the *ramose* form of inflorescence is considered as a reversion to a more primitive and possibly ancestral type, its complete sterility in hybrids with the nearest wild relative of maize may be not without significance. The sterile inflorescences, while not succulent, suggest the sterile cauliflower form found in crosses between *ramose* and *podded* strains.

The combination of the teosinte form of pistillate inflorescence with the branched condition of *ramose* was completely sterile, and there seems to be little reason to believe that such a combination represents an ancestral form.

The *ramose* form of inflorescence, like the *crinkly* type of plant, was found to be associated with some of the characters of maize not involved in the change from normal to *ramose* inflorescences, thus furnishing additional evidence that there is a correlated complex of maize characters which tends to reappear in the perjugate generations of hybrids with teosinte. The *ramose* form, however, is correlated with only three maize characters, and two instances of apparent disherence are found.

The brachytic segregates of the  $F_2$  teosinte hybrid resembled the normal segregates much more closely than has been found to be the case in brachytic-normal maize hybrids; and there seems little doubt that modifying factors affecting stature have been introduced through the teosinte parent. Only 12 per cent of the plants of the second generation were classified as brachytic.

As in the two preceding characters, brachytic culms are found to be associated with other maize characters; and, while the correlations often are not high, it seems clear that somewhat the same complex of maize characters is involved as in the other two hybrids.

All three characters, *crinkly*, *ramose*, and brachytic, are found to be correlated with other characters of maize, but in many cases the characters involved differ in the three hybrids. Thus the *crinkly* type of plant shows a high correlation with short season, as indicated by the negative coefficients with days to pollen and days to silk, while neither the *ramose* nor brachytic plants show correlations with season. Similarly, *crinkly* is associated with few leaves, while the other two characters show no such association. Both *crinkly* and brachytic plants have few suckers, but the *ramose* plants seem not to have this limitation.

It seems clear that many of the multiple-factor characters of maize are correlated with these three unit characters, but the characters involved and the degree of closeness of their relations differ in the three hybrids.

The close relationship of maize and teosinte is further emphasized by these hybrids and, with the exception of the *ramose* type of branching, there is little evidence of incompatible combinations.

On the other hand, the cross with brachytic maize indicates that teosinte possesses modifying factors for this character not present in at least a large number of maize varieties. This same condition was indicated also by the hybrids with golden and rainbow.

It is further shown that the branching of the ramose variation, which would seem to represent a reversion to a more primitive form, does not approximate the branched inflorescence of teosinte, but is farther removed from teosinte than from normal maize.

With respect to the correlations among the characters which differentiate maize and teosinte, but which are not involved in the change from normal maize to the three variations studied, there seems to be practically complete freedom of recombination within the limits of physiological relationships.

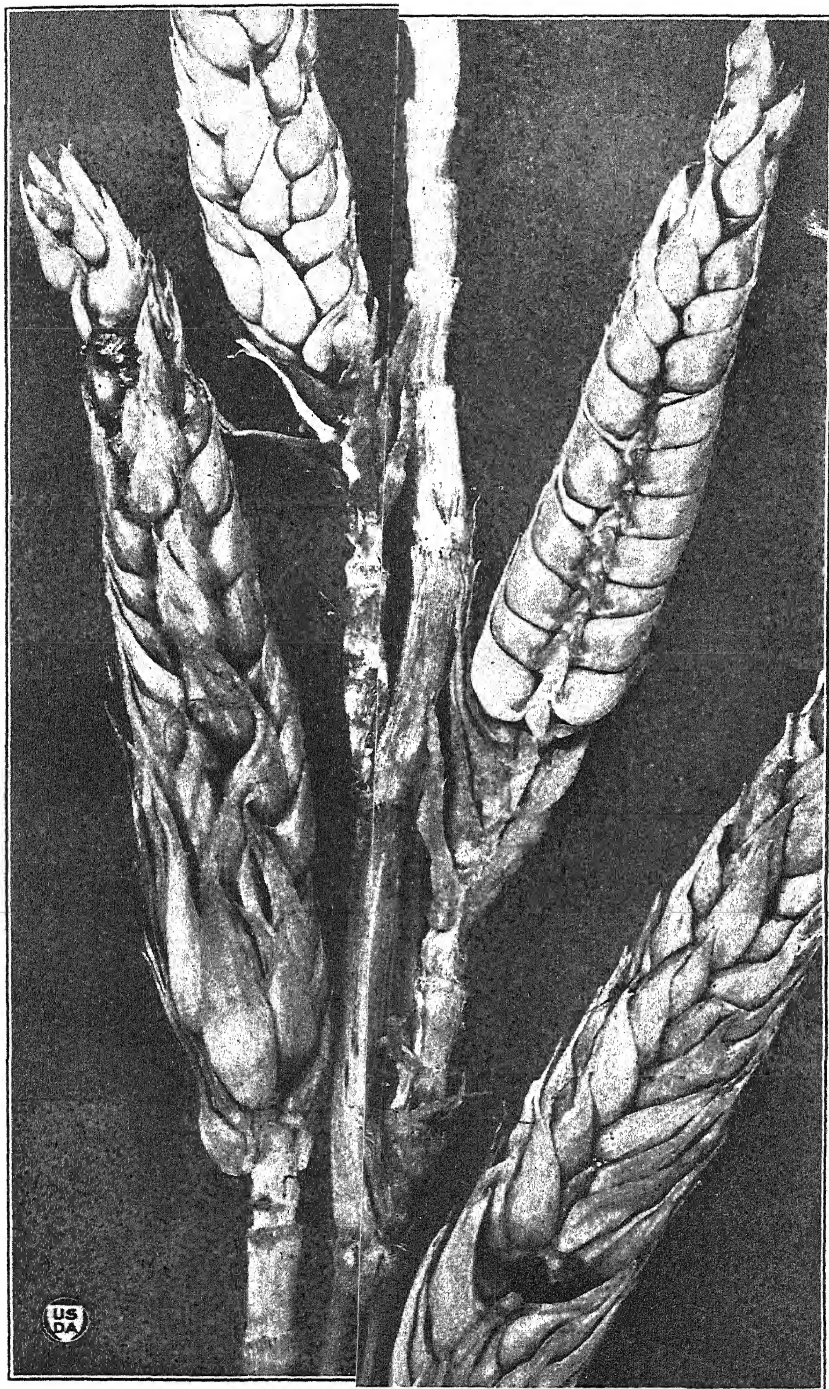
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PLATE I

Lateral inflorescence from a plant of the first generation of a cross between pod corn and teosinte. The husks have been removed to show the character of the spikes. Natural size.



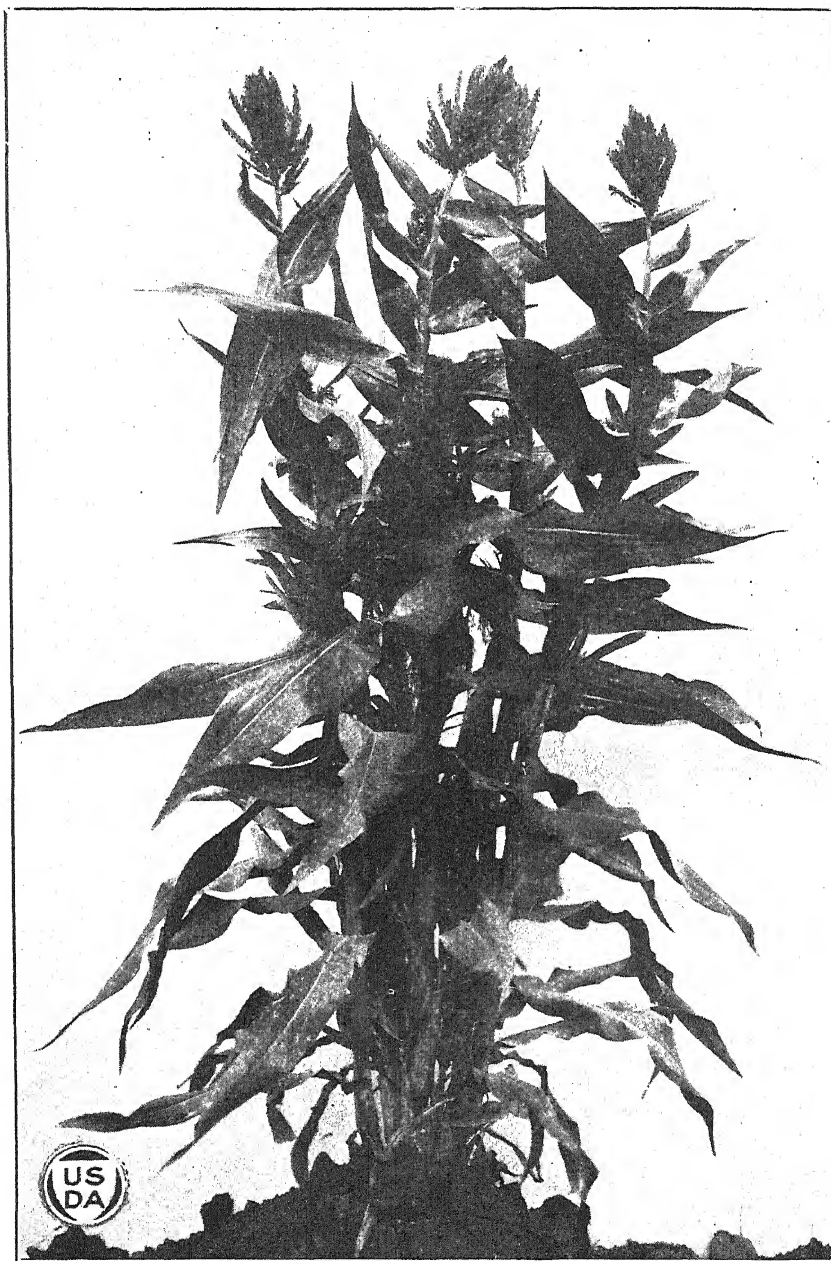


PLATE 2

Plant of the second generation of crinkly maize  $\times$  teosinte. This plant exhibits many of the characteristics of crinkly maize.

PLATE 3

Plant of the second generation of crinkly maize  $\times$  teosinte. This plant, in contrast to that shown in Plate 2, exhibits many of the characteristics of teosinte.





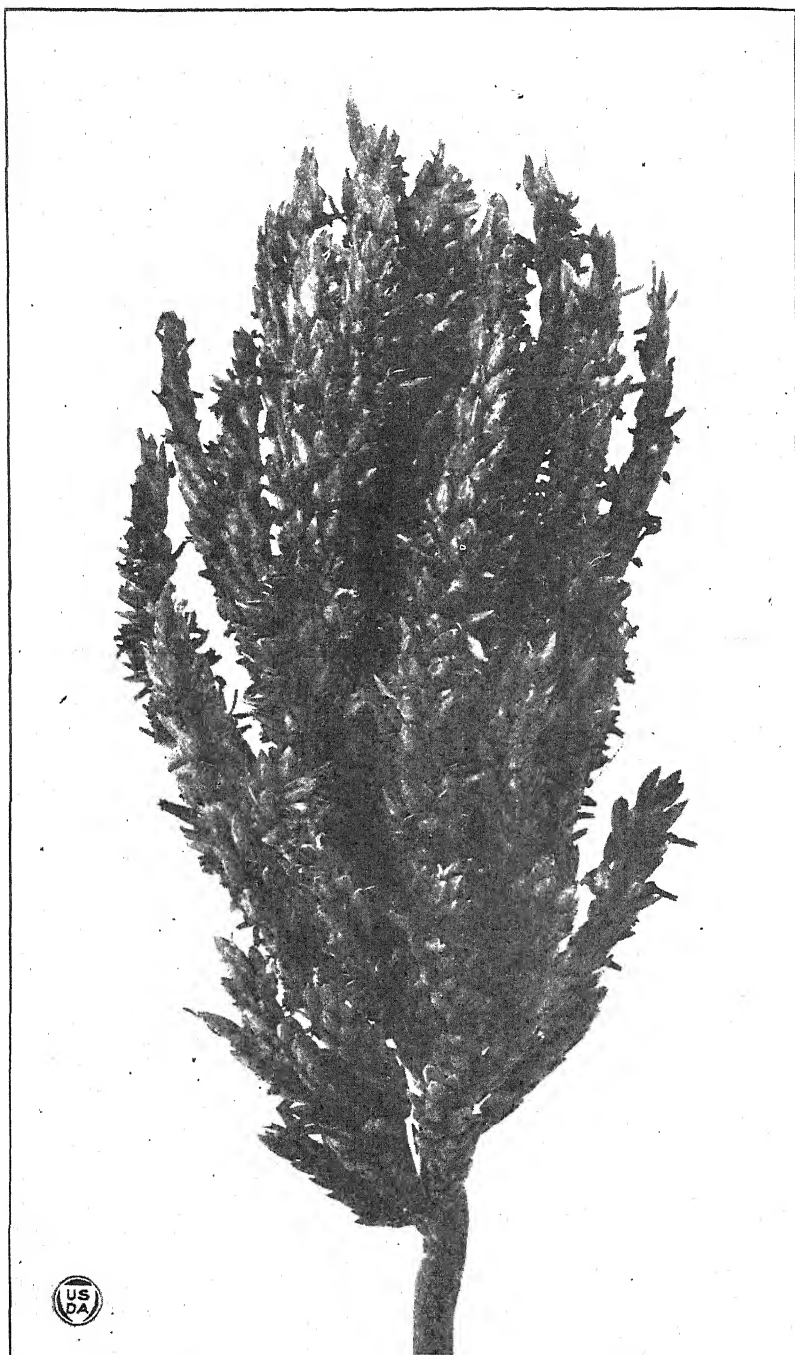


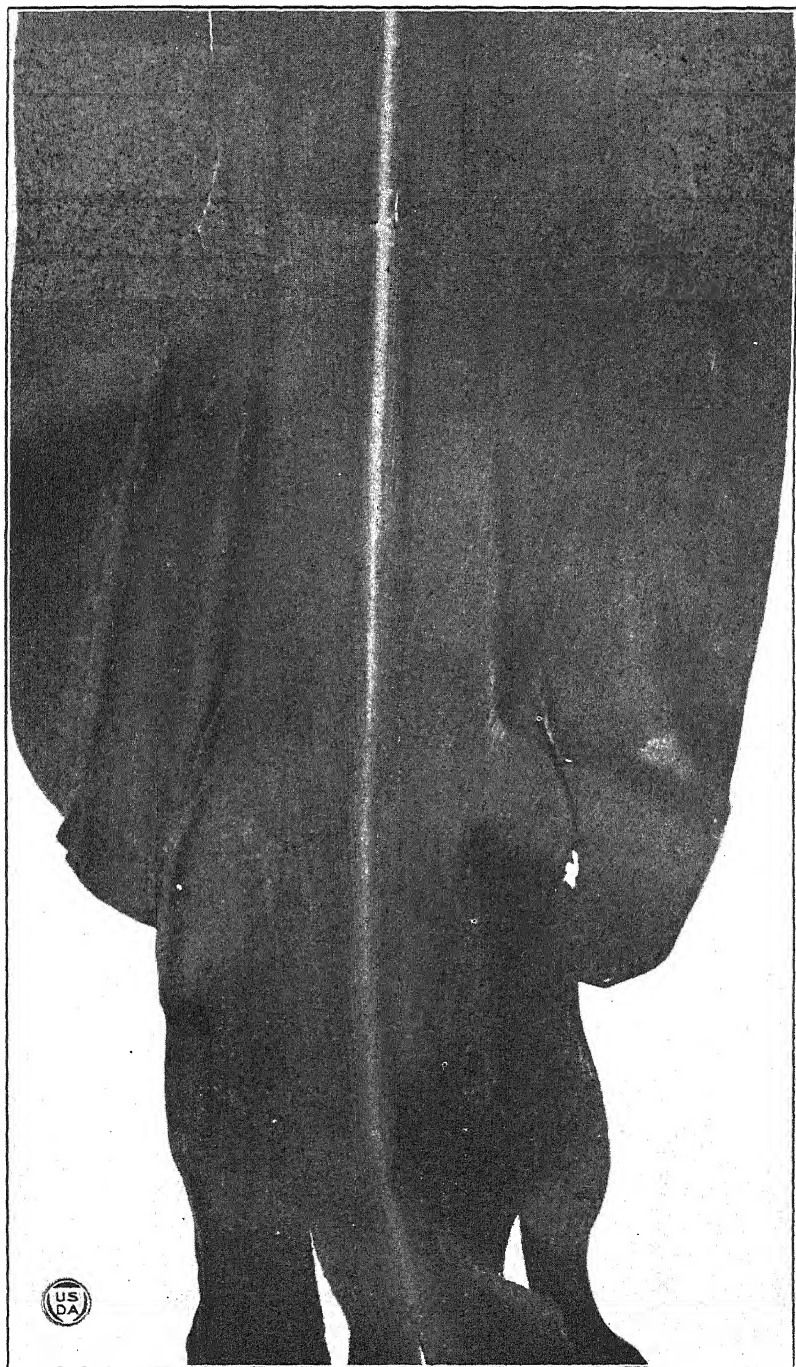
PLATE 4

Terminal inflorescence of the plant represented in Plate 2, showing the compact type of tassel typical of crinkly maize. Natural size.

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PLATE 5

Section of a leaf from the plant shown in Plate 2 showing pronounced lobing at the base. About natural size.



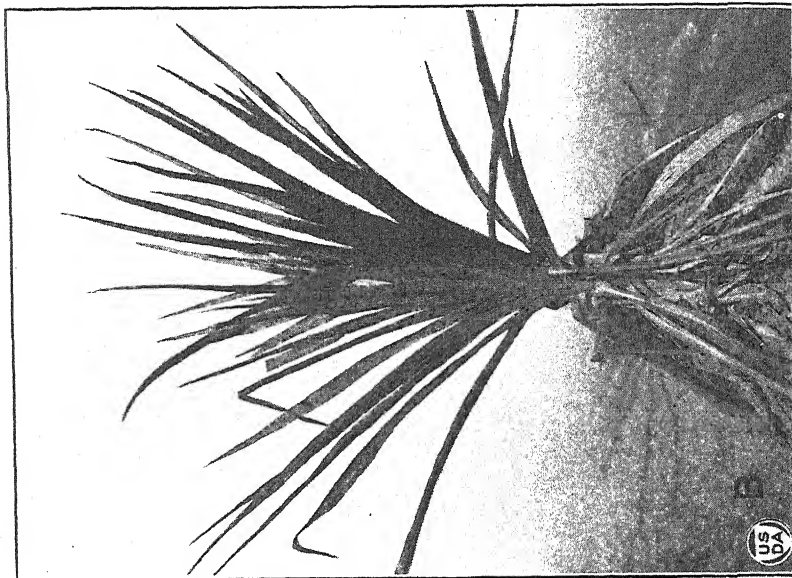


PLATE 6

A.—A ramose plant from the second generation of ramose maize  $\times$  teosinte. Note the typical ramose tassel.

B.—A spike-leaved brachytic plant from the second generation of brachytic maize  $\times$  teosinte.

#### PLATE 7

A.—Lateral inflorescence from the plant represented in Plate 6, A. The husks have been removed to show the character of the spikes.

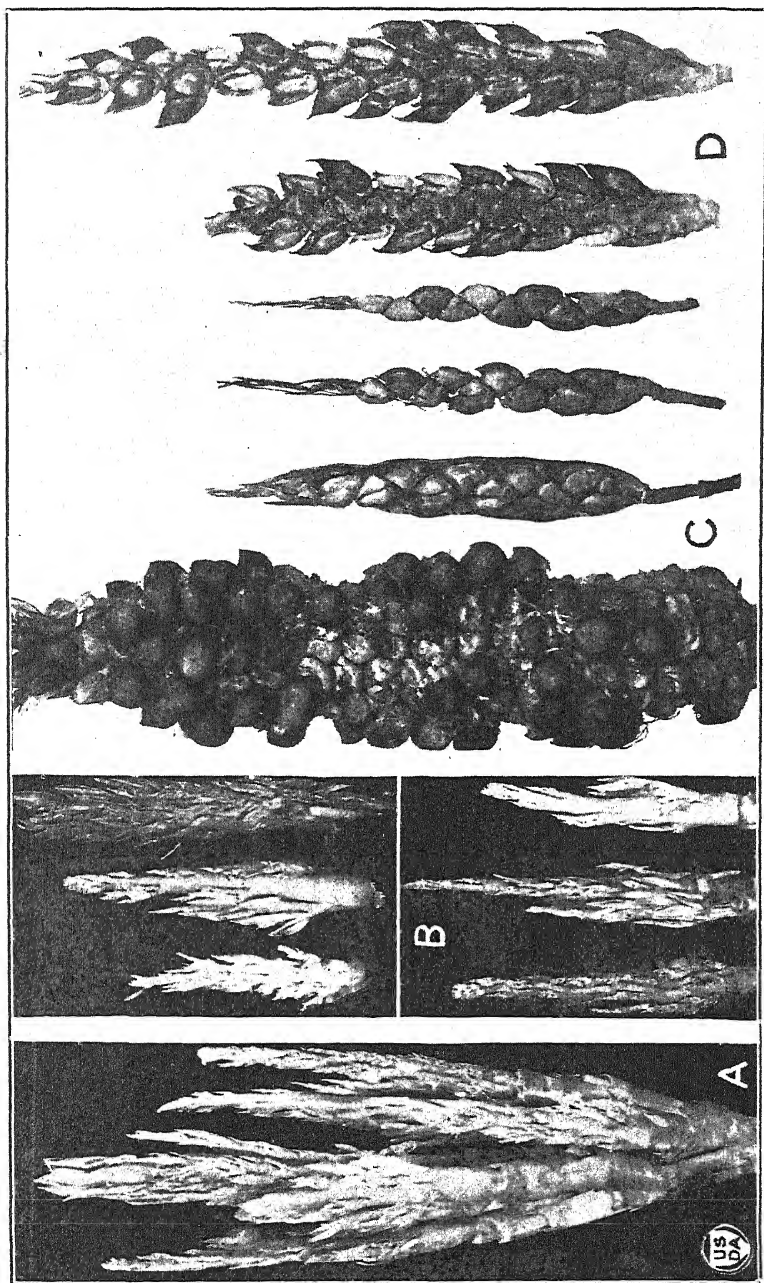
B.—Individual spikes from the prophyllary branch of the inflorescence shown at the left in A.

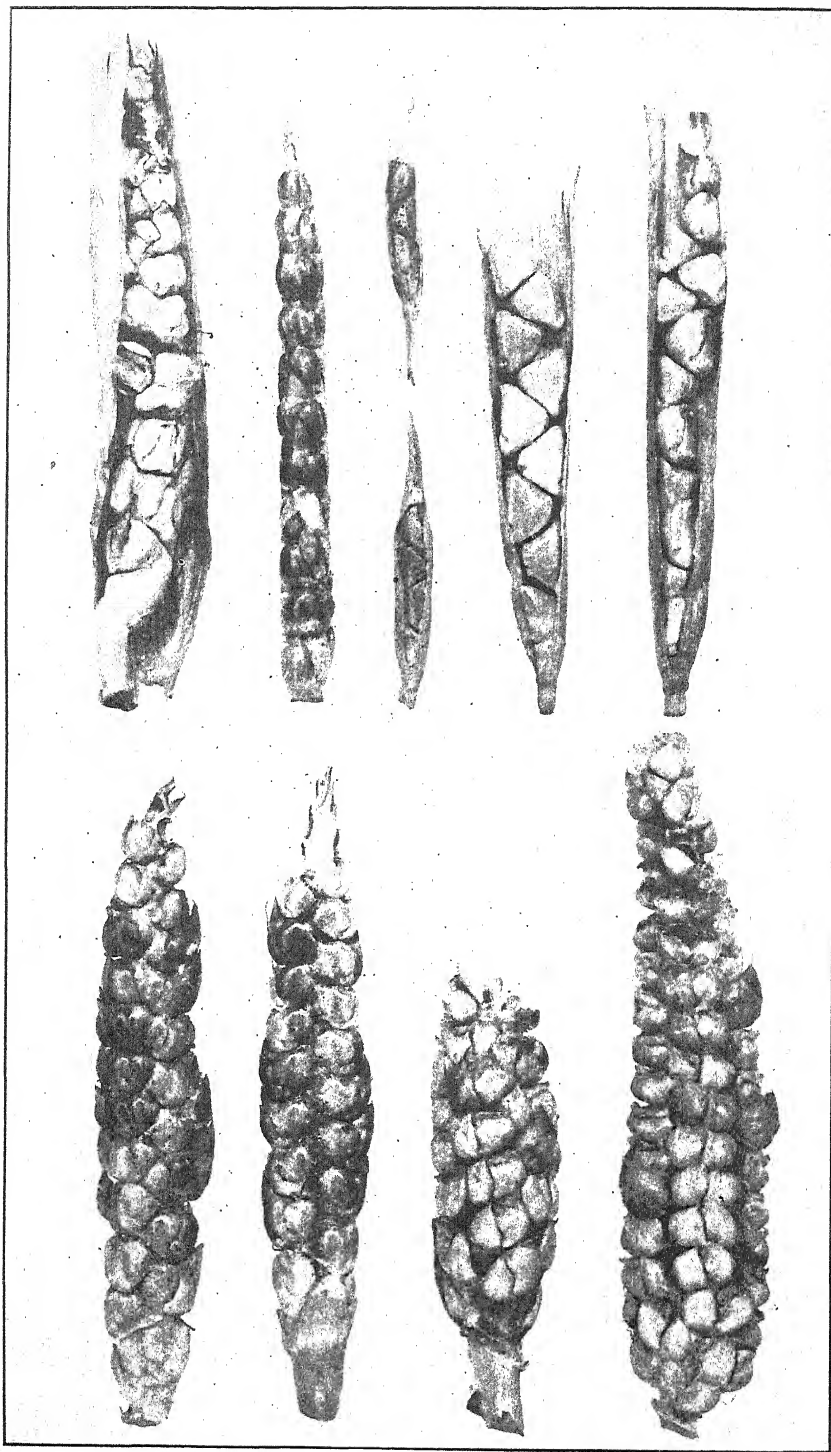
C.—The most maizelike and most teosintelike inflorescences obtained in the second generation of the ramosa maize  $\times$  teosinte hybrid. The teosintelike seeds resemble the triangular seeds of Durango teosinte rather than the seeds of the Florida teosinte parent. Natural size.

D.—Spikes from the second generation of brachytic maize  $\times$  teosinte, showing extreme form of beaked seeds. Natural size.

Beaked seeds were a characteristic of one of the great-grandparents of the brachytic maize, but were not noticeable in the immediate brachytic plant which served as the female parent of the brachytic-teosinte hybrid.







#### PLATE 8

Spikes from the second generation of brachytic maize  $\times$  teosinte. The two spikes at the upper right show the gigantic seeds resembling in form Durango teosinte. The two spikes in the upper center are the most teosinte like recovered in maize-teosinte hybrids and have seeds which resemble in form the seeds of the Florida teosinte parent though much smaller in size. The four spikes at the left (2 upper and 2 lower) are from a single plant of the  $F_2$  and represent very closely inflorescences typical of  $F_1$  plants. The upper left-hand spike is terminal and shows the "saddle-back" condition encountered in maize-teosinte crosses.

The two spikes at the lower right are the most maize like inflorescences found in the  $F_2$  of brachytic maize  $\times$  teosinte.



# THE UTILIZATION OF LACTOSE BY THE CHICKEN<sup>1</sup>

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The value of milk, in its various forms, as a poultry feed has been clearly established, the high quality of its proteins and minerals being its chief assets. The present investigation was undertaken primarily for the purpose of determining whether or not lactose, the carbohydrate of milk, was utilized by the chicken. It is not always realized that lactose represents approximately 38 per cent of the total solids and approximately 30 per cent of the energy of whole milk, while the total proteins contain but 26 per cent of the solids and approximately 20 per cent of the energy of whole milk. In some commercial milk preparations the percentage of lactose is much higher; whole milk powder contains 34 to 41 per cent, skim milk powder 48 to 54 per cent, and secwa, or dried whey, contains approximately 74.5 per cent lactose and 14.2 per cent soluble albumin (5).<sup>3</sup> From a practical point of view the carbohydrates of a ration usually seem of less importance than the protein because of the lower relative cost of the former, but a knowledge of the amount and utilization of the carbohydrates present is necessary in order to formulate the most desirable and economical ration for a particular purpose. Thus a knowledge of the amount and degree of utilization of the lactose in milk is of practical importance to the science of poultry feeding. The question is also of theoretical interest because at no stage in the life of the chicken is milk, or any other substance containing lactose, a part of its natural diet, and therefore the utilization of lactose, which normally requires the presence of a lactase, might rightfully be questioned on teleological grounds.

The literature available on the subject of the utilization of lactose by the fowl is very meager. In addition to investigations on the adaptation of various digestive organs to lactose, only two studies have been found dealing directly with the question. Shaw (7), in a study of digestion in the chick, concluded that lactose was not digested and, further, that it acted as an irritant to the gastrointestinal mucosa. Chicks fed from birth on milk alone died on the third day and the contents of no part of the intestinal tract gave a positive test for monosaccharids with Barfoed's reagent.

That lactose was present was shown by preparing the phenyllactosazone crystals. Plimmer and Rosedale (6), on the other hand, claim to have fed chickens from birth to a period exceeding three months on a diet containing lactose. Failure to find reducing sugars in the excreta was taken to indicate the assimilation of lactose.

## EXPERIMENTAL DATA

In determining the utilization of lactose, the reducing sugars present in the excreta of hens fed on an ordinary cereal diet and on a diet containing variable amounts of lactose were estimated. Seven experiments,

<sup>1</sup> Received for publication Jan. 26, 1924.

<sup>2</sup> The writers desire to thank Dr. H. H. Mitchell for many helpful suggestions and advice regarding certain procedures followed in this work. They are also indebted to C. I. Bray for some preliminary analytical work in connection with the sugar methods used.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 604.

each of one week's duration, were made. In all experiments in which lactose was fed, except Experiment No. 2, the hens received the lactose with their mash on each of three successive days. The excreta were collected daily during these three days as well as during the four following days in order to allow sufficient time for the excretion of any unabsorbed lactose. Between successive experiments four days were ordinarily allowed to elapse.

In Experiment No. 2 two grams of lactose were fed daily to each hen for seven days. The excreta were collected on the seventh day only. Four mature White Wyandotte hens were used in this investigation. Each hen was kept in a wire cage 15 inches wide, 24 inches long, and 14 inches high. The wires of the cage were 2 inches apart one way and 4 inches apart the other. In the bottom of the cage was the excreta-collection pan, which was of the same width and length as the cage and 1.5 inches deep. It rested on flat iron grooves which held the pan firmly in place but which permitted removal for cleaning. The pan was made water-tight, of medium weight galvanized iron. Inside of the pan was an iron frame covered with hardware cloth. This frame was just enough smaller than the pan to fit closely within it and was raised about 1 inch from the bottom. The hen stood on this frame and the excreta for the most part soon passed through the hardware cloth into the pan below. In this manner the excreta were neither trampled over nor scratched out and lost. The feeding and watering jars were supported outside the cages and protected in such a manner that the hen was unable to scatter feed into the collection pan. The hens seemed contented and appeared not to mind in the least the comparatively close quarters in which they were confined.

During each experiment the collection pans were cleaned daily. An extra collection pan was available and was substituted for the regular pan while the latter was being cleaned. In cleaning the pan, the frame was first freed from any excreta remaining on it by scraping the excreta into the pan below. The pan itself was then thoroughly cleaned with a steel spatula. The excreted material from each hen was kept at 0° to 4° C. in an air-tight, half-gallon glass fruit jar until the end of the experiment, when it was analyzed. In order to preserve the excreta, a 1:1 mixture of alcohol and water, containing 10 per cent thymol, was poured over the frame each day after cleaning, the excess being caught in the pan. After running the solution over all parts of the pan, the excess was drained into the jar containing the excreta. A small amount of powdered thymol was also sprinkled over the excreta in the jars.

The procedure adopted in this investigation depended upon the fact that the excreta from chickens fed on a cereal diet normally contain little, if any, reducing sugars. This was shown by Brown (3) who carefully examined, by the phenyl-hydrazine test, the excreta of chickens fed on a corn diet. No trace of an osazone could be detected. Plimmer and Rosedale (6) found no reducing sugars in the excreta of chickens even when fed on a diet containing lactose. In the present investigation the results obtained indicate that there is no constant excretion of reducing sugars in chickens fed on a cereal diet. The determination of the completeness of utilization of ingested lactose, therefore, consisted in the analysis of the excreta for reducing sugars. In all cases the quantitative analysis was substantiated by a qualitative examination of the excreta for sugars by the formation of osazones and their identification under the microscope.

The procedure adopted for the extraction of the reducing substances in the excreta was as follows: The total excreta of each hen for the period of the experiment were extracted by pouring 200 to 500 cc. portions of hot water over the excreta in a large evaporating dish, macerating thoroughly with a pestle, and filtering through cloth. The residue in the cloth was returned to the dish and extracted again. When the total volume of the washings measured 4,000 cc., the extraction was considered complete. An extract of this volume was previously shown to be sufficient by mixing 25 gm. of lactose and 25 gm. of glucose with a seven-day collection of excreta and extracting with 200 to 500 cc. portions of hot water until the last extract failed to give a carbohydrate test with Fehling's solution. The reducing substances in the total excreta for the period of the experiment were estimated by determining the reducing substances, calculated as lactose,  $C_{12}H_{22}O_{11}$ , in aliquots of the thoroughly mixed extract.

Two methods were used throughout for the determination of reducing substances. The first was the combination of the Munson and Walker and the Bertrand (*z*) methods, as described by Mathews (4, p. 994), for the quantitative determination of reducing sugars in urine and other fluids, and the second was Benedict's (*r*) method for the estimation of glucose in urine. The first is a method of the Association of Official Agricultural Chemists<sup>4</sup> for the determination of reducing sugars in foods and feeding stuffs and has been found in this laboratory to be rapid and accurate. This method, however, has the disadvantage that substances other than sugars, uric acid in particular, are capable of effecting reduction of the alkaline cupric tartrate and hence, if present, would be calculated as lactose. Uric acid would, of course, be present in considerable quantities in hot aqueous extracts of chicken excreta. However, since the quantity of uric acid excreted daily from a diet in which only the amount of lactose varied would probably change but slightly, it was thought that the variation in quantity of reducing sugars present could be estimated without the previous removal of uric acid. On the other hand, the Benedict method is not appreciably affected by the presence of uric acid. Tests designed to show the effect of uric acid and to determine the applicability of the combined Munson-Walker-Bertrand and the Benedict methods to aqueous extracts of chicken excreta containing a constant amount of uric acid and varying amounts of lactose were conducted.

First, the effect of uric acid on the determination of lactose in milk by each of the two methods was tested. Lactose was determined in a sample of separated milk by the two methods in the usual manner. Then, uric acid, varying from 0.01 gm. to 1 gm. was added to 25 cc. portions of the sample and the lactose was determined by the combined Munson-Walker-Bertrand and by the Benedict methods in the usual manner with the following averaged results.

<sup>4</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to November 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

TABLE I.—*Effect of uric acid on the determination of lactose by the Munson-Walker-Bertrand and the Benedict methods*

## PER CENT OF LACTOSE IN SEPARATED MILK

Uric acid added.	Munson-Walker-Bertrand method.	Benedict method.
<i>Gm.</i>		
.....	4. 145	4. 152
.....	4. 106	4. 156
.....	4. 176	.....
.....	4. 192	.....
	<sup>a</sup> 4. 155	<sup>a</sup> 4. 154
0. 01	4. 200	4. 157
0. 10	4. 400	4. 150
0. 90	5. 475	4. 161
1. 00	.....	4. 173

<sup>a</sup> Average.

It is readily seen from Table I that uric acid markedly affects the estimation of lactose by the combined Munson-Walker-Bertrand method. In fact, where 1 gm. of uric acid was added to a 25 cc. portion of separated milk it was impossible to carry out the test. That the Benedict method is unaffected by uric acid to any appreciable extent is also shown.

In a second test about 1,200 gm. of combined excreta from four hens, fed on the standard cereal diet, were mixed thoroughly and four portions of 200 gm. each removed. To each of two portions, 25 gm. of lactose were added and the samples mixed. All four samples were then extracted in the same manner with four 300 cc. portions of hot water. Each extract was made up to 1,500 cc., mixed, and lactose determined in aliquot portions by both sugar methods in the manner in which they are used in urine analysis. The results have been averaged and summarized in Table II. Table II also contains the averaged results obtained when 1 and 2 gm. of lactose were added to excreta in a manner similar to that when 25 gm. were added. The reducing substances were determined by the Benedict method only in these cases, however.

TABLE II.—*Recovery of lactose added to excreta*

Sample of lactose.	Total reducing substances calculated as lactose.			
	Munson-Walker-Bertrand method.		Benedict method.	
	I.	II.	I.	II.
<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>	<i>Gm.</i>
0	0. 34	0. 35	0. 03	0. 04
25	25. 81	26. 21	24. 86	25. 13
1	( <sup>a</sup> )	( <sup>a</sup> )	1. 08	1. 11
2	( <sup>a</sup> )	( <sup>a</sup> )	2. 30	2. 21

<sup>a</sup> Not determined.



These tests indicated that for the purpose of this investigation either method could be used with a fair degree of accuracy. Both methods were employed throughout and the technique used was the same as that recommended for each method when applied to urine analysis. In order to obtain additional data and also to serve as a check on the quantitative results, the phenyl-hydrazine test was uniformly applied to all extracts. The osazones were examined microscopically only.

TABLE III.—Summary of results on the utilization of lactose by the hen

Experiment No.	Daily diet.	Hen No.	Duration of experiment.	Presence of osazones in excreta.		Total reducing substances excreted calculated as lactose, $C_{12}H_{22}O_{11}$ .	
				Lactos-azone.	Glucos-azone.	Munson-Walker-Bertrand method.	Benedict method.
			Days.			Gm.	Gm.
I	Basal ration plus 6 gm. lactose	I	.....	—	—	1.98	1.70
		2	7	—	—	0.80	0.64
		3	.....	—	—	1.21	0.78
		4	.....	—	—	1.57	1.84
II	Basal ration plus 2 gm. lactose	I	.....	—	—	0.00	0.16
		2	I	—	—	0.00	0.03
		3	.....	—	—	0.00	0.03
		4	.....	—	—	0.00	0.15
III	Basal ration.....	I	.....	—	—	4.34	1.34
		2	7	—	—	3.29	1.40
		3	.....	—	—	0.63	0.60
		4	.....	—	—	1.54	0.72
IV	Basal ration plus 18 gm. lactose.	I	.....	—	+	5.52	3.89
		2	7	—	+	0.08	3.78
		3	.....	+	+	5.16	4.08
		4	.....	+	+	10.84	7.78
V	Basal ration.....	I	.....	—	+	3.08	0.56
		2	7	—	+	2.03	1.33
		3	.....	—	+	3.71	1.68
		4	.....	—	+	2.52	1.05
VI	Basal ration plus 24 gm. lactose.	I	.....	+	+	3.08	1.79
		2	7	+	+	8.16	5.36
		3	.....	+	+	6.11	3.52
		4	.....	+	+	9.37	7.07
VII	Basal ration.....	I	.....	—	+	2.38	0.70
		2	7	—	+	3.01	1.26
		3	.....	—	+	2.73	0.98
		4	.....	—	+	3.15	1.61

## DISCUSSION

The basal ration consisted of 30 gm. of a mixture of oats and cracked corn, morning and evening, and 30 gm. of moistened mash at noon. The mash consisted of equal parts by weight of wheat bran, flour middlings, ground corn, ground oats, and tankage. The lactose fed during all the experiments was Merck's U. S. P. lactose monohydrate which proved

to be 99 per cent pure by both the Munson-Walker-Bertrand method and the Benedict method. It was fed with the mash at noon. The results are reported in grams of lactose excreted during the experiment. As would be expected from the fact that the determination of reducing sugars is affected by uric acid in the Munson-Walker-Bertrand method, while this acid does not affect, appreciably, the Benedict method, the results obtained by the latter method are, with few exceptions, much lower than those obtained by the former. On a comparative basis, however, the differences between experiments are as well shown by the one method as by the other. In the preparation of the osazones for the identification of individual sugars in the extracts the utmost care was taken, for this method of distinguishing between the sugars has never proved to be infallible in this laboratory, and especially was this found to be the case with extracts such as those dealt with in this investigation. The purified free base was used since it was found to give more satisfactory results than the hydrochlorid. While the qualitative osazone tests were not quite as satisfactory as might be desired, they confirm very well the quantitative data. In general, they indicate that lactose does not appear in the excreta of hens until comparatively large amounts are fed, that the glucosazone usually is found whenever the lactosazone is present, and that glucose persists in the excreta for some time after the lactose has disappeared.

In feeding lactose to hens it was immediately noticed that amounts exceeding 2 gm. daily caused diarrhea, a finding in agreement with that of Shaw (7). In order to test the possibility of the diarrhea itself being the cause of an increase in the excretion of reducing substances, two experiments, in which diarrhea was caused by feeding Epsom salts ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) in the drinking water, were made. The results indicated clearly that the diarrhea was not the cause of the excretion of comparatively large amounts of reducing substances when lactose exceeding 2 gm. was fed.

The results of seven experiments, summarized in Table III, show that pure lactose when added to a cereal diet is utilized to a large extent by hens. All hens excreted more or less reducing substances when fed a normal cereal diet; this was, of course, expected. The amounts of reducing substances excreted by the four hens in three seven-day experiments on the lactose-free diet varied from 0.56 gm. (calculated as lactose) to 1.68 gm. by the Benedict method, as indicated in the following summary:

Hen No.	Experiment.			Average.
	III.	V.	VII.	
	Gm.	Gm.	Gm.	Gm.
1.....	1.34	0.56	0.70	0.87
2.....	1.40	1.33	1.26	1.33
3.....	0.60	1.68	0.98	1.09
4.....	0.72	1.05	1.61	1.13
Average.....				1.10

Since the method of extraction was always the same, that is, the number of extractions and the quantity and temperature of the water were exactly the same in all cases, it follows that the amounts of reducing substances excreted by each hen during the different weekly periods on the ordinary cereal diet were not constant. The average amount of reducing substances excreted by each hen during seven days was, by the Benedict method, 1.10 gm., and by the Munson-Walker-Bertrand method, 2.70 gm., expressed as lactose. If, therefore, we use these average figures as representing the grams of reducing substances originating from the basal ration in the extract of any seven-day collection of excreta, the percentages of lactose absorbed, when varying quantities of lactose were fed, may be calculated as shown in Table IV.

TABLE IV.—Percentage absorption of lactose

Hen No.	Percentage of lactose absorbed when the following quantities were fed—							
	2 gm.		6 gm.		18 gm.		24 gm.	
	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.	Benedict method.	Munson-Walker-Bertrand method.
1.....	100	100	90	100	85	85	97	93
2.....	100	100	100	100	85	82	83	77
3.....	100	100	100	100	84	87	90	86
4.....	100	100	88	100	63	55	75	73

Table IV is self-explanatory; it shows that hens will utilize lactose up to 8 gm. daily fairly completely. Attempts to feed larger quantities failed because the hens would not voluntarily eat their mash when more than 8 gm. had been mixed with it. Even when 8 gm. were fed daily it was necessary to force-feed much of the mash on the third day to all hens. This was probably due to the severe diarrhea caused by this quantity of lactose when fed in this form. Because of the severe diarrhea it was not considered wise to attempt to feed larger amounts.

The difficulties experienced in these experiments in inducing the chickens to consume large quantities of pure lactose when mixed with the feed, and the severe diarrhea always obtained when even 8 gm. a day were consumed, are in striking contrast to the experience of Plimmer and Rosedale (5). These investigators fed a diet containing secwa (a dried whey preparation containing 74.5 per cent lactose) to chickens from birth for a period exceeding four months. The amount of secwa in the diet was usually 25 per cent but in some cases was even higher. The quantity of lactose consumed in this form by each bird was as high as 22 gm. daily. Good gains were obtained and the health of the birds was excellent throughout the experiment. No reasonable explanation of these differences occurs to us.

## SUMMARY

Lactose, up to 8 gm. per hen per day, was utilized fairly completely, thus making it evident that the lactose present in the quantity of whole milk, skim milk, whey, or buttermilk normally consumed (100 to 200 cc.) by chickens would be completely absorbed.

When lactose appears in the excreta, it is usually accompanied by glucose.

Lactose when fed mixed with a moist mash acts as an irritant to the gastrointestinal mucosa. Chickens will not voluntarily consume more than about 8 gm. of lactose daily when fed in the form of pure lactose mixed with the feed.

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# THE OCCURRENCE OF LACTASE IN THE ALIMENTARY TRACT OF THE CHICKEN<sup>1</sup>

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## INTRODUCTION

In the experiments reported in the preceding paper (p. 597) it was found that lactose, fed to hens, does not appear in the excreta in appreciable quantities until excessive amounts are administered—a finding thoroughly in agreement with our present knowledge of sugar-feeding in human beings. Since lactose is assimilated from the alimentary canal of chickens, and since assimilation of disaccharids is normally preceded by hydrolysis into monosaccharids, lactase would be expected to be present in some part of the alimentary tract. However, the possibility of bacterial fermentation accounting for the disappearance of lactose must be considered.

Previous investigations are not entirely in agreement in regard to the presence of lactase in the alimentary tract of the fowl. Portier (4<sup>2</sup>) in 1898, using the osazone method for the detection of monosaccharids formed by the action of the enzyme extracts on a lactose solution, found no indication of lactase in the intestines of birds. This was confirmed a year later by the extensive investigations made by Weinland (6) who, using the polarimetric method, found no indication of lactase in the intestines of fowls. He states, however, that the enzyme is produced in the intestines of fowls if they are fed on milk and lactose. Bieri and Portier (7), using the duck in their experiments, found in one case, on feeding lactose and milk, that the intestines produced lactase, but a second experiment failed to confirm the first. Plimmer (2), in an extensive investigation on the subject of adaptation of the intestines to lactose, found that the intestines of fowls are naturally free of lactase and, furthermore, that lactase was not produced when lactose and milk were fed for long periods of time. Plimmer used Allihn's reduction method for the estimation of the degree of hydrolysis of a lactose solution produced by the enzyme extracts. In a recent study on the distribution of enzymes in the alimentary canal of the chicken, however, Plimmer and Rosedale (3) found lactase to be present in the crop, but absent in the proventriculus and intestines. The presence of the enzyme was determined by incubating at 37° C. a 4 per cent lactose solution with portions of boiled and unboiled extracts of the various organs for two days or more, removing the proteins, and determining the reducing sugars by reduction of Fehling's solution. The observed difference in reading between the boiled and unboiled solutions indicated whether or not hydrolysis by lactase had occurred. In the present investigation a similar study has been made. This inquiry did not extend, however, to the detection of lactase in the

<sup>1</sup> Received for publication Jan. 26, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 608.

glandular structures in the floor of the mouth nor to the gizzard, the interior lining of which resembles parchment, making the presence of secretory glands improbable.

#### EXPERIMENTAL DATA

The method of preparing the enzym solutions was in accordance with those usually employed. The chicken was killed either by dislocating the neck or by decapitation, and the alimentary canal immediately removed. The crop was cut open, washed out with water, and, after being cut into small pieces, was ground in a mortar with sand and a little toluene to prevent putrefaction. The entire pancreas was ground in a similar manner with sand and toluene. The proventriculus and intestine were cut open, washed out with running water, and the mucous membranes scraped off and ground in a mortar with sand and toluene. The organs from three to six chickens were used in each of three experiments. In order to control more carefully the methods used in this investigation, the small intestines of five young rats were examined in the same manner as were those of the chickens. The extracts in all cases were made by adding 75 to 125 cc. of water to the ground organs and allowing the mixture to stand at room temperature for one to three days, with occasional shaking. The mixture was then strained through cloth to remove the sand and larger pieces of the organs and the filtrate was made up to a definite volume, usually 100 or 150 cc. The presence of lactase in the extracts was determined by adding to exactly 50 cc. portions of 4 per cent lactose solution, 25 cc. of the unheated extract in one case, and, as a control, 25 cc. of the boiled extract. Three cc. of toluene were added to each flask and the flasks corked and incubated at 36° to 38° C. for two to six days. After the period of incubation, the contents of the flasks were examined for monosaccharids by means of Barfoed's copper acetate reagent. This test was made without previous precipitation of the proteins. The digested extracts were carefully filtered and the clear filtrates used. The procedure adopted consisted in adding 2 cc. portions of the boiled and unboiled digested filtrates to 5 cc. portions of the copper acetate reagent and placing the resulting solutions in boiling water for exactly five minutes. The test was considered negative if no reduction was apparent during the five minutes boiling or during the three minutes after removal from the water bath. All tests were run in duplicate and the solutions containing the boiled and unboiled extracts were run side by side, at the same time and in exactly the same manner. The results obtained are summarized in Table I.

TABLE I.—*Distribution of lactase in the alimentary tract of the chicken*

Part of the alimentary tract used.	Number of chickens from which organs were com- posited.	Number of experiment.	Barfoed's test for monosaccharids.			
			Unboiled sample.		Boiled sample.	
			I.	II.	I.	II.
Crop.....	3	1	+	+	—	—
Do.....	3	2	+	+	—	—
Do.....	6	3	+	+	—	—
Proventriculus.....	4	1	—	—	—	—
Do.....	3	2	—	—	—	—
Do.....	6	3	—	(?)	—	—
Pancreas.....	4	1	—	—	—	—
Do.....	3	2	—	—	—	—
Do.....	6	3	—	—	—	—
Intestine.....	4	1	(?)	(?)	—	—
Do.....	3	2	—	—	—	—
Do.....	6	3	—	—	—	—
Small intestines from five rats.....		4	+	+	—	—

## DISCUSSION

Roaf (5) has shown that Barfoed's copper acetate reagent can be used with accuracy in testing the hydrolysis of disaccharids to monosaccharides by enzymes if a boiled control is run at the same time and in exactly the same manner as the unboiled solution. Preliminary experiments with this reagent and with two quantitative reduction methods, the Munson-Walker-Bertrand combination and the Alihn method, indicated that the qualitative copper acetate reagent could be used most accurately and advantageously with this type of solution. The chief disadvantages of any quantitative method lie in the fact that the difference in the reducing power of lactose and of hydrolyzed lactose is small. Slight errors in measurement of solutions might easily make consistent differences in the amount of reduction, which might indicate hydrolysis. When the lactose is only partly hydrolyzed, the difference in reduction would be still less, and when the sugar solution is very dilute the results obtained by the quantitative reduction methods can not be considered satisfactory. Examination of the table will show uniformly negative reductions in all lactose solutions containing the boiled enzyme extract. This is a further check on the accuracy of the method, the absence of reduction in these tubes proving that any reduction taking place in the unboiled extract tubes was due only to the hydrolytic action of an enzyme and not to any other treatment such as the reaction of the solutions or boiling.

As shown by the table, the unboiled extracts of the crop showed an unquestionable reduction of the copper acetate reagent and, consequently, the presence of lactase. The unboiled extracts from neither the proventriculus, the pancreas, nor the intestines of the chickens gave any signs of reduction, with the exception of three questionable results, which are indicated in the table by question marks. These results were questioned because, while a very slight brownish coloration was obtained, it was not sufficient to be considered a positive test, and at the same time was different from the corresponding boiled extracts. Compared with the

amount of reduction obtained in the case of the unboiled extracts of the small intestines of young rats, the unboiled crop extracts of chickens showed considerably less reduction. These results were an unexpected confirmation of the results of Plimmer and Rosedale (3), who found lactase in the crop of chickens but in no other part of the alimentary tract.

#### SUMMARY

Lactase was found to be present in the crop but absent in the proventriculus, the pancreas, and the intestines of normal chickens.

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# THE LIFE HISTORY OF THE GRAPE ROOTROT FUNGUS *ROESLERIA HYPOGAEA* THÜM. ET PASS.<sup>1</sup>

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## INTRODUCTION

The fungus *Roesleria hypogaea*, associated with a rootrot of grapes, has long been known, especially in the grape-growing regions of Europe. It has not been definitely proved that this fungus is the primary cause of the rot, but there is no question that, once established, it contributes largely toward the killing of the roots. Disagreement among mycologists as to the identity of some well-known fungus is often due to a lack of knowledge of its life history. Confusion has more than once arisen because of an accidental similarity of fruit bodies or spores. A case in point is that of *Roesleria* and *Pilacre*.

We are indebted to Brefeld (5<sup>2</sup>) for the beautiful illustrations of *Pilacre petersii* B. & C., a fungus which he believed to represent a very primitive Basidiomycete. His figures of the fruiting bodies on bark show a "gleba" composed in part of short septate branches, each bearing a definite number of spores. Such a branch he considers a simple basidium. *Roesleria hypogaea* Thüm. and Pass., an ascogenous fungus whose fruiting bodies have a strong superficial resemblance to those of *P. petersii*, is, on this account, most interesting. *Calicium pallidum* Pers., well known to lichenologists, also has fruiting bodies similar in general appearance to those of *Roesleria* and *P. petersii*. Since Rehm (27, p. 396) considers *Coniocybe pallida* (Pers.) Fr. a synonym of *R. hypogaea*, and Bayliss-Elliott and Grove (8) suggest that *R. hypogaea* is merely the ascogenous stage of *P. petersii*, and since Viala and Pacottet (35) report conidial and chlamydosporic forms of *Roesleria* in culture, it is not surprising that we find the greatest confusion of these names in the literature. The writer has had the opportunity recently to study *R. hypogaea* and *P. petersii* in culture and has also compared them with specimens of the lichen, *Coniocybe pallida*, with the result that we find that there is no basis for considering these fungi identical or genetically related.

From a review of the literature it will be seen that the grape rootrot fungus has been described under a number of different names and combinations. The following list includes such as appear from original descriptions and illustrations to refer to a Discomycete identical with *Roesleria hypogaea* of von Thümen and Passerini.

*Pilacre subterranea* Wein. 1832. (36, p. 458.)

*Pilacre friesii* Wein. (in Flora) 1832. (36, p. 458.) Not Wein. 1834.

*Onygena friesii* Wein. 1834. (37, p. 413-414.)

*Vibrissina flavipes* Rab. 1852. (26, p. 286.)

*Sphinctrina coremioides* B. & Br. 1872. (2).

*Roesleria hypogaea* Thüm. & Pass. 1877. (32.)

<sup>1</sup> Received for publication Nov. 23, 1923.

<sup>2</sup> Reference is made by number (italic) to "Literature cited, pp. 615-616."

*Vibrissea hypogaea* (Thüm. & Pass.) Rich. 1881. (28.)

*Coniocybe pilacriiformis* Rehm 1892. (20, p. 56.)

*Roesleria pilacriiformis* (Rehm) Henn. 1895. (16.)

*Pilacre pilacriiformis* (Rehm) Boud. 1907. (4.)

*Pilacre pallida* Boud. 1907. (4.) Not *Calicium pallidum* Pers. 1794 (23, p. 20), nor *P. pallida* E. & F. 1900. (9, p. 59.)

A lichen *Calicium pallidum* Pers. 1794 (23, p. 20), (*Coniocybe pallida* (Pers.) Fr. 1824 (11, I, p. 3), has also been confused with *Roesleria hypogaea*. The specific name "pallida" has been used frequently in combination with the generic name *Roesleria* (*R. pallida* (Pers.) Sacc. (30, p. 299) to refer to a fungus thought at the time to be one of the Stilbaceae but which is in reality *Roesleria hypogaea*.

Another fungus, nonascogenous, commonly called *Pilacre faginea*, or *P. petersii* in this country, has been regarded erroneously by various authors as a conidial stage of *Roesleria hypogaea*. It appears to have been first described as *Onygena faginea* Fr. The synonymy of this fungus so far as can be ascertained by a study of original descriptions and the literature is as follows:

*Onygena faginea* Fr. 1818. (10, p. 25.)

*Onygena decorticata* Schwein. 1822 (31, p. 65.) Not *Onygena decor-ticata* Pers. 1799. (24.)

*Phleogena faginea* (Fr.) Link 1833. (21, p. 396.)

*Pilacre friesii* Wein. in Linnaea. 1834. (37, p. 413-414.) (Not *P. friesii* Wein. in Flora 1832. (36, p. 458.)

*Botryochaete faginea* (Fr.) Corda 1854. (7, pl. 9, fig. 95.)

*Pilacre faginea* (Fr.) B. & Br. 1850. (3, v. 5, p. 365, pl. 11, fig. 5.)

*Ecchyna faginea* Fr. 1857. (13, p. 151.)

*Pilacre petersii* B. & C. 1859. (3, v. 3, p. 362.)

*Stilbum pilacriiforme* Rich. 1889. (29), not *Coniocybe pilacriiformis* Rehm (20, p. 56).

The grape rootrot fungus has been reported growing on the roots of various hosts such as *Vitis*, *Malus*, *Pyrus*, *Cydonia*, *Prunus* (almond and cherry), *Salix*, *Tilia*, *Rosa*, and *Paliurus*.

From a résumé of the literature, it would seem that the advocates of the parasitic nature of *Roesleria* as opposed to its saprophytic nature of growth are almost evenly divided. The following consider it parasitic: Lemonnier (19), d'Arbois de Jubainville (1), Hennings (16), Jolicœur (17, p. 219-224), Massee (22, p. 289), and Bayliss-Elliott and Grove (8). Gillot (14), von Thümen (33, p. 210-212), and Prillieux (25) believe it to be somewhat parasitic, while it is thought to be nonparasitic by Berkeley (2), Cooke (6), Laurent (18), Viala and Pacottet (35), Hartig (15, p. 83), and Verge (34). The results of the writer's experiments show that when ascospores are sown in wounds the fungus can establish itself in living roots.

### CULTURES

Cultures were made on various media from ascospores from the fruiting bodies of *Roesleria* on apple roots (Pl. 1, A) collected in New York City in October, 1920, by Dr. Dodge.<sup>3</sup> Plates of cleared corn-meal agar were

<sup>3</sup> DODGE, B. O. A ROOT-ROT DISEASE OF APPLE SEEDLINGS. (Title) In Amer. Assoc. Adv. Sci. Program. 71st meeting, p. 32. 1918. A number of French crab-apple seedlings, obtained through a nursery, had been grown in pots for two years, then set out in the garden. During this time the plants had been inoculated with the pear-blight organism, and had been attacked somewhat by wooly aphids in the garden. Early in November in 1917 it was noticed that several of the little trees were falling over, due to the fact that their root systems had been destroyed by some rot. By digging down in the soil a few inches, partially decayed pieces of roots were found bearing numbers of ascocarps of *Roesleria*. The greenish mycelium of the fungus was discovered at least a foot beneath the surface of the ground. Fruiting bodies of the fungus were found on roots of plants the remainder of the root systems of which appeared to be perfectly healthy.

inoculated on October 18, 1920, with ascospores from the fruiting heads. In two days numerous spores germinated. Growth was slow, the germ tubes having reached a length of not more than  $20\mu$ . Viala and Pacottet (35) described fairly accurately the method of germination, showing that a few spores became septate during germination but more of them were undivided. Single spores were marked in the plates, and when they had germinated each spore was transferred to a tube of slanted corn-meal agar. All of the writer's cultures discussed in this paper were derived by transfer from these single ascospore cultures. Eight single spore cultures were made and kept at room temperature until the mycelium had made good growth, and they were then placed in a refrigerator where the temperature averaged 10 to  $12^{\circ}\text{C}$ . This provided uniformly cool, moist and dark conditions for growth.

#### CORN-MEAL AGAR CULTURES

On the corn-meal agar the fungus grows slowly forming a thin layer of felty mycelium, white to buff at first, becoming green at the center, the green color gradually spreading over the surface. It becomes blackish green with age, sometimes taking on a grayish tinge. None of these first transfers fruited, but later transfers were made to the same medium, making in all 28, and at the end of five months stalked fruiting bodies were found on three cultures, one culture having three at the base of the agar. These were small and white or grayish white ascocarps with heads scarcely wider than the stalks. The heads contained asci and paraphyses which were slender and extended out beyond the asci.

#### OATMEAL PASTE AGAR CULTURES

Agar in which the nutrient medium is an oatmeal paste was found very satisfactory for the development of *Roesleria*. Forty-five transfers were made at different times from single ascospore cultures to tubes of this medium; 37 of these were kept at least part of the time in the refrigerator at  $10^{\circ}$  to  $12^{\circ}\text{C}$ . The mycelium spreads very slowly, forming a compact growth, cream or buff colored at first which becomes a bright malachite green, darkening with age. The growth is more felty, more luxuriant, and of a brighter green than that on the corn-meal agar. In from five to seven months ascocarps appeared in a large number of the tubes. They occurred singly or in groups of from 2 to 15 (Pl. 1, B, F, G). The stalks were white to grayish with mouse-gray heads. These fruiting bodies,  $4$  to  $4\frac{1}{2}$  mm.  $\times$  1 mm. were somewhat larger than those occurring in nature on the roots, the stalk being thicker and the heads larger, 3 mm. wide in the largest ones.

#### CULTURES ON APPLE ROOTS

Transfers were made from each of the single-spore cultures to autoclaved apple roots in large test tubes, with a few cubic centimeters of water in the bottom. These cultures were kept in the refrigerator at  $10$  to  $12^{\circ}\text{C}$ . The fungus grew well, covering the roots with a felty or fluffy mycelium, white to buff at first, later becoming bright malachite or fluorite green (Ridgway).<sup>4</sup> In from 6 to 12 months, when the cultures had dried somewhat, fruit bodies containing mature asci appeared

<sup>4</sup> RIDGWAY, Robert.—COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

singly or more often in groups (Pl. I, C, E). As many as 14 ascocarps developed in one group. The larger ascocarps developed to maturity, but some of the smaller ones had not matured by the time the cultures dried.

#### CULTURES ON RUBUS STEMS

Four similar tubes containing blackberry stems with a few leaves were sterilized, inoculated, and treated in the same manner as previously described for apple roots. The mycelium which developed was light to dull green and felty but less luxuriant than on the apple roots. The cultures fruited sparingly, one having several small ascocarps on the midrib of a leaf, another with several fruiting in different stages of development at the base of the stem. Even the smallest ascocarps matured.

#### CULTURES MADE FOR COMPARISON WITH THOSE OF VIALA AND PACOTTET

In view of the fact that in the writer's cultures described above ascocarps were developed without the fungus producing any intermediate spore forms such as the conidia reported by Viala and Pacottet (35), she has endeavored to grow the fungus in such a way as to duplicate as nearly as possible the cultures described by them. The writer's methods were as follows: Two 2-liter flasks were filled to a depth of 6 or 7 cm. with kidney-bean juice with  $\frac{1}{10}$  per cent tartaric acid and 5 per cent sugar added. (The fungus did not grow on media made with 1 per cent tartaric acid as specified by Viala and Pacottet.) These were steamed for three successive days. They were inoculated with bits of mycelium from single ascospore cultures. No. 1 was kept in the light at room temperature averaging 20 to 25° C., No. 2 was treated in the same way for five months, then put into a refrigerator with temperature at about 10 to 12° C. About a week after inoculation, one colony grew on the surface of each and within a day or two showed a greenish color. These colonies soon appeared warty, raised at the edges, and depressed in the center, which was yellowish green. Around the colonies the liquid was iridescent, forming a film. A month or so later the colonies appeared heavy and fell to the bottom. In flask No. 1 the colony grew considerably and formed numerous rounded protuberances covered with whitish mycelium, giving them a fluffy appearance. Some of the nodules were as large as 1 to 1½ cm. in diameter. The main part of the colony was dark, with some brownish accretions or precipitate from the liquid. Several months later flask No. 2, which had been in the refrigerator for some time, showed 12 or 15 immersed colonies, while the other flask had only 3 or 4. Some had greenish to deep-green zones, while one or two appeared deep green and warty only at the center. None of the colonies adhered to the glass, as was the case in Viala and Pacottet's cultures, but all remained separate and free in the liquid. They were subspherical or hemispherical, but had no stalks such as they describe. Microscopically, all showed mycelium with numerous rounded subhyaline swellings, often occurring singly or in rows of 3 or 4. The mycelium was of a slightly yellowish or yellowish-brown tinge. Viala and Pacottet called these bodies chlamydospore fruits, and the rounded swellings chlamydospores. It is a well-known fact that the mycelium of many species of fungi which grow for a long time in culture becomes abnormal, and the cells become misshapen so as to resemble chlamydospores. Viala and Pacottet did

not show that they function as spores in any way. While the writer did not find these bodies quite so regularly developed in long chains as described by these authors, there can be no doubt that they are identical.

One 2-liter flask of kidney-bean juice with 2 per cent agar and pieces of steamed grapevine added was autoclaved and inoculated with bits of mycelium from a single-spore culture. In a few days compact round colonies buff in color appeared. These increased in size and when about one-half inch in diameter were raised in the center, felty, and pale green with rim of white or buff. The green color deepened and brightened, and when the colonies were 5 cm. across they were deep grayish green with wide white or cream colored margins. The colonies coalesced with distinct lines of demarkation. Soon concentric rings appeared with the centers glaucous, green, and felty. The other rings were in order, buff color and white, the newest growth being white. The whole growth was superficial, and no fruiting bodies of any kind were ever formed.

Another 2-liter flask of kidney-bean juice containing 4 per cent agar with  $\frac{1}{10}$  per cent tartaric acid and 5 per cent sugar with pieces of steamed grape vine added was inoculated in the same way as the other flasks. This medium had a jelly-like consistency. Seven or eight compact felty buff-colored colonies appeared in less than two weeks, one showing a faint greenish-brown tinge. Two or three weeks later the colonies were quite irregular, felty, and greenish yellow, with wide white or buff colored margins, which soon coalesced. No fruiting bodies or chlamydospores were formed on this medium. Viala and Pacottet report that their cultures on similar media produced conidia.

#### INOCULATION OF GRAPE ROOTS

A number of roots of grape were inoculated in April, 1921, by placing mycelium in wounds made on the roots or by spraying them with ascospores produced in culture. A number of fruiting bodies were found eight months later on certain roots which had been sprayed with ascospores. These fruiting bodies were characteristic ascocarps, except that some of them had greenish stalks and heads, while some were buff colored with cinder-gray heads. They produced asci and ascospores of the usual sort.

#### DISCUSSION

The results of the culture experiments show that ascocarps were formed on all the media in tube cultures after four or five months, 37 cultures fruiting. None has been found in the large flasks of kidney-bean media, but these cultures comprised a much larger bulk and remained in a moist condition, and up to the time reported on were not dried down, as were the test-tube cultures. Ascocarps were developed abundantly in cultures from single ascospores and without any intermediate spore form such as *Pilacre petersii* or other imperfect stage. The best medium for the production of fruiting bodies was obtained by autoclaving apple roots in test tubes. These were large tubes giving a good supply of air, and when the culture medium had dried somewhat fruiting bodies formed in abundance. Rubus stems used in the same way proved much less satisfactory, while oatmeal-paste agar was second only to apple roots, and these cultures could be depended on to produce numerous ascocarps. A cool, humid atmosphere, such as obtains in the ordinary refrigerator, was apparently essential to the production of fruiting bodies. At room temperature in diffuse light only one culture was found with ascocarps.

Viala and Pacottet (35) report that they had no success whatever in germinating ascospores in culture. They used ascospores which germinated on heads developed from the old mycelium in roots kept several months in damp chambers. This method would open the way for contamination. The writer's cultures were all obtained from single ascospores germinated in Petri dishes and transferred each to a tube of nutrient agar; later cultures were obtained by transfer from these. Viala and Pacottet found no difference in the color of the mycelium on the different media used, all having shown a malachite green. The writer found this green color fairly constant except on the kidney-bean media to which tartaric acid and sugar were added. In this medium a slight green color was observed in the early stages of growth, but for the most part a greenish-yellow color obtained, noticeable particularly on the solid medium and to some extent in the liquid medium. The only yellow color mentioned by Viala and Pacottet was in connection with what they thought were conidiophores, which they noted were white to yellowish in the early stages, and their "chlamydosporic fruits" which were yellow when young. The conidiophores described by them in some of their older cultures have not been observed in the writer's cultures at any time, although some of them are more than two years old. As for the chlamydosporic fruits of Viala and Pacottet, structures with such appearances are often found in old cultures of fungi and have no significance.

#### SUMMARY AND CONCLUSIONS

Whether the name *Pilacre* should be applied to an ascomycete or not is impossible to say until the nature of Fries' specimen of *P. weinmanni* is known (12). Without such an investigation, just as pointed out by Bayliss-Elliott and Grove (8), any attempt to settle definitely the question of priority with regard to the names which have been applied to the grape and apple rootrot fungus would be premature.

The *Roesleria* of von Thümen (32) is an ascomycete which can be grown easily in culture from ascospore to ascospore. The spores are sometimes septate on germination, producing one or two germ tubes. This fact has led some to suggest that it belongs in the family Geoglossaceae. A fine septate felty mycelium is formed, which both in culture and frequently on the roots shows a characteristic malachite green. Ascocarps are formed in culture in the refrigerator, where the dark, cool conditions simulate the natural soil conditions where ascocarps mature in the fall of the year. Notwithstanding the fact that ascocarps were frequently formed only after the agar had dried out considerably, this dryness is clearly not a necessary condition, because fruit bodies were formed sometimes on the web of hyphae floating on the water in the bottom of the test tubes containing sterilized roots which were used instead of an agar medium (Pl. 1, D). The strain from apple rootrot is evidently the same as that found on grape, since inoculations of grape roots with the strain from apple have resulted in the formation of similar ascocarps.

In discussing the relationship between *Pilacre* and *Roesleria*, Bayliss-Elliott and Grove (8) state:

Moreover it became evident that it would do no violence to the facts if it were concluded that *Pilacre faginea* and *P. petersii* were also identical with each other, and that both resembled the *Roesleria* so much in character as to make it seem not unlikely that *Pilacre* is only a stage of *Roesleria*.

And later:

The conclusion at which we have arrived is that *Pilacre* is a conidiophorous fungus, not in any sense a Basidiomycete, and that it is not in the remotest degree allied to the Auriculariaceae and Tremellineae, but is a stage of the Discomycetous genus *Roesleria*. This suggestion can no longer be entertained. No conidial stage has ever appeared in the life cycle of *Roesleria* which the writer has grown to maturity many times in single ascospore cultures. Conidia are produced freely in cultures of the Basidiomycete *Pilacre petersii*. *Roesleria* is hypogaeous, developing large quantities of ascospores which can not be discharged into the air in any way comparable to that prevailing in most other Ascomycetes, the asci deliquesce allowing the spores to mass together in the head, which is at first covered with a peridium-like web of hyphae. This is soon broken away by the crowding of the spores. Spore distribution is probably brought about by disturbances of the soil by insects, earthworms, or by cultivation.

It has been pointed out that there are three distinct fungi having fruit bodies which are more or less similar in appearance: One, a lichen growing on bark, *Calicium (Coniocybe) pallidum*; another, a Discomycete growing on roots, *Roesleria hypogaea*; and third, *Pilacre petersii*, the primitive Basidiomycete of Brefeld. If *Pilacre* is an ascogenous genus, *P. petersii* does not belong with it. Certainly *R. hypogaea* is not a lichen. There is no basis for considering either *Coniocybe pallida* or *Pilacre petersii* synonymous with *Roesleria hypogaea*.

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PLATE I

A.—Ascocarps of *Roesleria hypogaea*, collected October, 1920, on root of French crab seedling grown in the garden of Columbia University, New York City. The writer's cultures were obtained from ascospores from this specimen.

B.—Culture of *Roesleria* on oatmeal paste in tube showing ascocarps, 10 months after inoculation. Natural size.

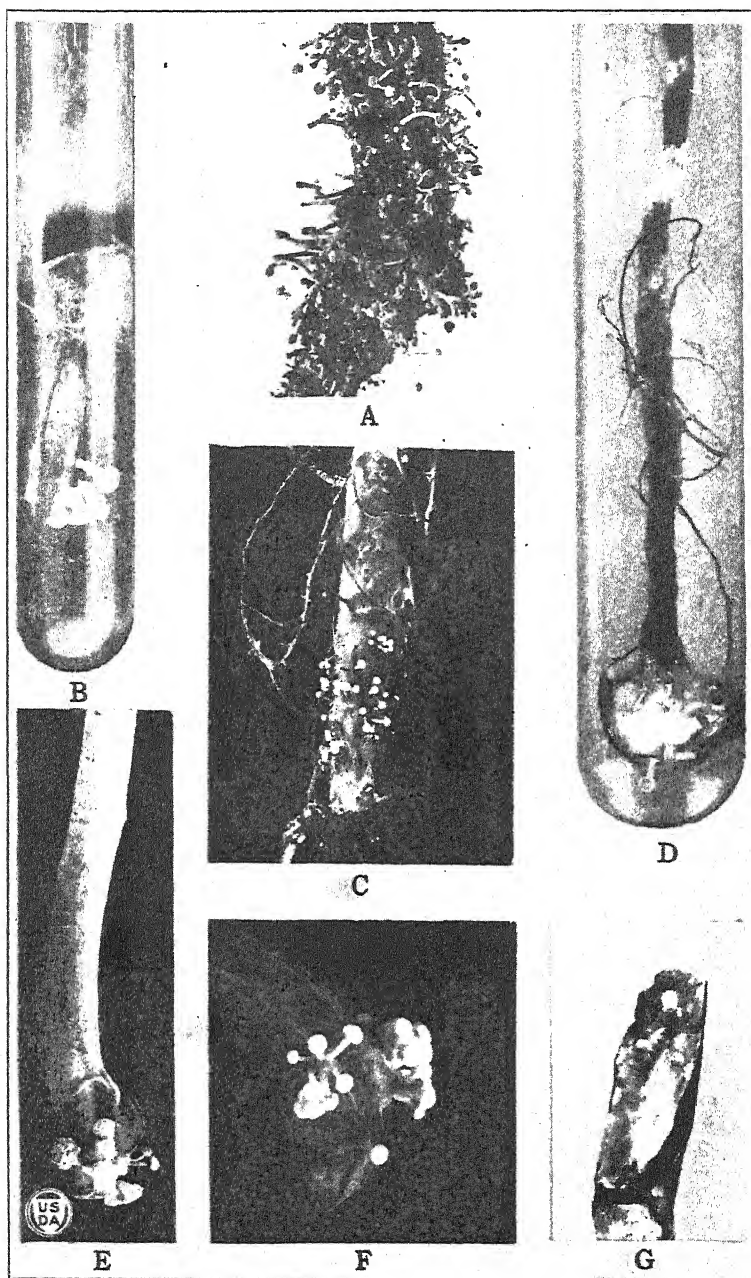
C.—Apple root from tube culture showing numerous small ascocarps, 9 months old. Slightly magnified.

D.—Apple root in tube showing ascocarps produced on the hyphal web formed on the water at the bottom. Culture scarcely 5 months old. Natural size.

E.—Apple root from tube culture showing a group of ascocarps somewhat older (9 months). The "peridium" in each has broken away.  $\times 1.4$ .

F.—Oatmeal paste agar culture removed from the tube showing groups of large ascocarps. Culture a year old.  $\times 1.4$ .

G.—Oatmeal paste agar culture removed from the tube showing ascocarps on upper part of slant. Culture 5 months old. Natural size.





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## THE MOVEMENT OF WATER IN IRRIGATED SOILS<sup>1</sup>

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### INTRODUCTION

Irrigation is a relatively new feature of agriculture in the United States. As yet there has not been time to accumulate a fund of experience in irrigation to correspond to our knowledge of farming under conditions of ample rainfall. We are now practicing irrigation under a great variety of conditions as to climate, soil, and character of water supply, and are gaining experience rapidly. Large investments have been made in irrigation works and in the improvements and equipment on irrigated land. These investments include not only money but even in larger measure the labor of the farmer and home maker, which have been applied in the belief that irrigation farming is no less permanent than farming under conditions of adequate rainfall. The structures installed for the storage and diversion of irrigation water have been built to last for generations. It is clearly the expectation that the lands to be served by this water will continue to be productive as long as the works shall stand. Yet our own experience, which covers but little more than half a century, shows that there is a real danger that some of our irrigated lands may become unproductive within a few years. The causes of this uncertainty as to the future are found in what is known as the alkali problem.

The present paper deals with certain aspects of the alkali problem which as a whole relates to the soluble salts in the soil solution. These salts, which are derived from the processes of rock decomposition and soil formation everywhere, do not accumulate in the soil except in arid regions where the evaporation exceeds the rainfall. In regions of abundant rainfall the soluble products of rock decomposition are continually leached away from the soil and carried to the sea.

In attempting to present an account of the alkali problem in relation to irrigation farming it seems desirable to consider first the physical relations existing between the water and the soil. An understanding of these relations is essential to a comprehension of the alkali problem. In an irrigated field the soil acts as a reservoir to hold water for the use of plants. As a reservoir it has definite limits of capacity, both as to the quantity of water it may hold within the zone occupied by plant roots and as to the time required to fill it with water. These limits and the factors which determine them need to be understood as clearly as our knowledge permits, both by the farmer who operates the land and by the engineer who designs and constructs the irrigation works.

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## SOIL WATER

In an irrigated field the soil water that is of chief concern to the farmer is the water that exists in the surface layer of the soil from 4 to 10 feet deep, depending on the character of the crop for which the field is used. This surface layer of soil serves as a reservoir in which to store water from time to time by irrigation, so that it may be continuously available to the crop plants during the growing period.

The water that is put into this surface layer of soil may not all be used by plants. On the one hand, if the soil is saturated with water, the roots of most crop plants do not function properly. If the roots have been established before the soil becomes saturated they may die below the level of saturation. If the soil is already saturated the roots do not penetrate the saturated zone. Consequently, a soil may contain too much water to support the growth of crop plants. This condition is not uncommon in irrigated lands, and such lands are referred to as "water-logged."

On the other hand, some of the water in the soil is not available to plants because it is held so firmly by the soil that the plants can not use it. When the moisture content of the soil is so low that plants can not obtain from it enough water to maintain their growth there still remains an appreciable quantity of water in the soil. It is customary to refer to the moisture condition of the soil at which plants can not obtain water for growth as the wilting point. It is only the water that is in excess of the moisture content at the wilting point that is available to crops or that constitutes the available reservoir capacity of the soil.

The upper limit of the reservoir capacity of the soil is a point well below the saturation point. This upper limit is commonly referred to as the maximum field carrying capacity. The maximum field carrying capacity of a soil is not susceptible of accurate determination because it is conditioned by forces that are constantly changing.

## THE WATER-HOLDING CAPACITY OF SOIL

It is generally assumed that the specific gravity of soil material is 2.65, so that if we could conceive of a cubic foot of soil so compressed as not to contain any voids it would weigh 165 pounds. As a matter of fact, as it occurs in the field, soil is found generally to range in weight from 75 to 105 pounds per cubic foot, with a few exceptional cases in which it weighs less or more than the figures given. These figures refer to the dry weight of the volume of soil.

If we assume that the soil material has a specific gravity of 2.65, then it is possible to compute the pore space in a volume of soil, e. g., a cubic foot, when this volume has various weights. Thus if 1 cubic foot of soil without pore space weighs 165 pounds and a cubic foot of soil with pore space weighs 85 pounds, then:

$$\text{Pore space} = \frac{165 - 85}{165} = 48.5 \text{ per cent,}$$

or a cubic foot of dry soil which weighs 85 pounds may be said to have 51.5 per cent of its volume occupied by soil material and 48.5 per cent of its space existing as voids. If this void space were filled with water, that is, if the soil were saturated, but without changing the volume, then the soil would be said to hold 35.6 per cent of water. That is to say, if a cubic foot of water weighs 62.4 pounds, the water which occupies 48.5

per cent of that cubic foot weighs 30.26 pounds. If the dry soil in this cubic foot weighs 85 pounds, then 100 pounds of soil under the same condition of saturation would hold 35.6 pounds of water.

In like manner it is possible to compute the pore space and the water-holding capacity in percentage of water to the dry weight of the soil for any given case where the volume mass of the dry soil is known. Table I shows these relationships for several different weights of soil, and these are presented graphically in figure 1.

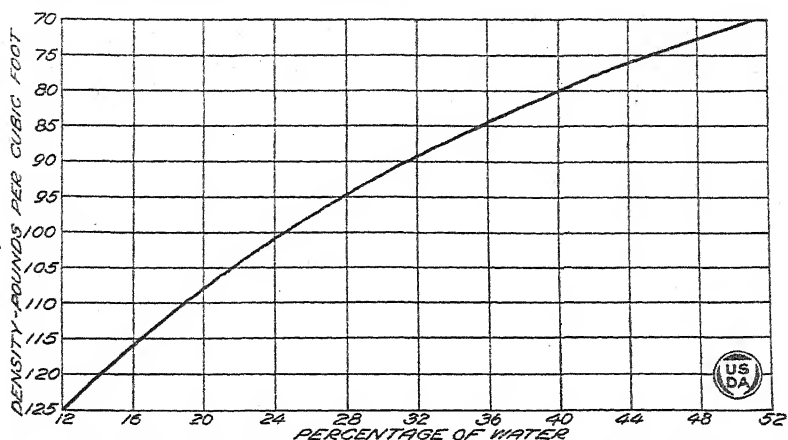


FIG. 1.—Diagram showing water-holding capacity of soil at densities ranging from 70 to 125 pounds per cubic foot, based on specific gravity of 2.65.

TABLE I.—Pore space in dry soil of different weights per cubic foot based on the specific gravity of 2.65 for the soil material and the moisture content if this pore space were filled with water

Weight of dry soil per cubic foot.	Pore space.	Water in saturated soil.	Water per foot of soil. <sup>a</sup>
Pounds.	Per cent.	Per cent.	Inches.
70	57.6	51.4	6.9
75	54.6	45.4	6.5
80	51.5	40.2	6.2
85	48.5	35.6	5.8
90	45.5	31.5	5.5
95	42.4	27.9	5.1
100	39.4	24.6	4.7
105	36.4	21.6	4.4
110	33.3	18.9	4.0
115	30.3	16.4	3.6
120	27.3	14.2	3.3
125	24.2	12.1	2.9

<sup>a</sup> By this is meant the equivalent, in inches in depth, of water contained in each foot in depth of soil.

It is not always an easy matter to determine the volume mass or the weight per cubic foot of the dry soil as it occurs in field conditions. The soil in the field is never entirely dry, and it may lose volume as well as weight when dried after being taken from its position in the field. It is this property of the soil by which it tends to increase its volume when

wet and to shrink on drying that constitutes one of the chief difficulties in the way of a clear understanding of its water-holding capacity. Soils differ greatly with respect to changes in volume that take place when they are alternately wetted and dried. Pure sand does not change its volume materially whether it is saturated with water or dry. Soil containing clay, on the other hand, swells extensively when wet and shrinks again when dried.

It is possible to demonstrate that a certain volume of pure sand retains its volume whether it is saturated with water or dry. If its volume mass is determined when it is wet and again when it is dry it will be found that the difference in weight between the dry condition and the saturated condition is substantially the same as the weight of the water required to fill the computed pore space. The computed pore space may range from 34 to 28 per cent, depending upon the assortment of sizes of the particles and the pressure to which the wet material has been subjected. The percentage of water occupying 34 per cent of pore space is approximately 19.5, while that occupying 28 per cent of pore space is about 14.6. The water which occupies or fills the pore spaces between the soil particles may be referred to as interstitial water.

When soil contains clay as well as particles of sand of assorted sizes its reaction with water becomes strikingly different from the reaction of pure sand. The addition of water to soil that contains clay tends to increase the volume of the soil mass. When the soil is not subject to pressure the increase in volume when wet may be very great. In the case of subsoil it is probable that the tendency to increase in volume on wetting is met by the pressures that develop within the soil mass. The quantity of water that may be absorbed by the soil some distance below the surface is very much less than the same soil will hold if it is at the surface.

These differences of water-holding capacity have been demonstrated by a series of tests made at the Newlands Experiment Farm by F. B. Headley.<sup>2</sup> Samples of subsoil were taken with a soil tube in places where free underground water was found within 2 to 3 feet of the surface. The soil samples were taken from below the level of the ground water. The moisture content was determined on the samples obtained with the soil tube, and the dried samples were then pulverized, sifted, and placed in metal cups of 20 cc. capacity having perforated bottoms. The soil in these cups was then saturated with water and weighed to determine its water-holding capacity in the absence of pressure. In texture the soil samples ranged from coarse sand to fine clay. For convenience in comparison they are arranged in Table II in groups with respect to texture. The results of these tests show that there are not only marked differences in the water-holding capacity of soils of different textures as they exist in the lower part of the root zone but that the water-holding capacity of the same soil is much greater when it is on the surface and freed from pressure than when it lies some distance below the surface and is subject to pressure.

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<sup>2</sup> These experiments were made at the writer's request during August, 1923.



TABLE II.—*Water-holding capacity of soil of different types, as found at 2 to 4 feet below the surface in the field and as determined in shallow perforated cups in the laboratory*<sup>a</sup>

Samples and texture of soil	Average water-holding capacity (percentage of dry soil)—		Increase.	Comparative increase. <sup>b</sup>
	In the field.	In the laboratory.		
No. 5, coarse sand.....	18.0	25.4	7.4	Per cent. 41
No. 8, fine sand.....	21.5	36.6	15.1	70
No. 7, sandy loam.....	20.7	41.5	20.8	100
No. 4, clay.....	33.5	78.7	45.2	135

<sup>a</sup> Reported by Headley from the Newlands Experiment Farm, 1923.<sup>b</sup> Stated as a percentage of the water-holding capacity of the sample in the field.

## THE WATER-HOLDING CAPACITY OF SUBSOIL

Some observations have also been made at Huntley, Mont., which show the water content of the subsoil just below and just above the upper limit of free underground water.<sup>3</sup> These were made in connection with the putting down of wells to be used for recording the fluctuations of the level of the underground water table during the irrigation season. In the early spring when these wells were put down the upper limit of the saturated zone was encountered between 9 and 12 feet below the surface.

The water content of the soil in the zone just below the level of the ground water is given in Table III. This shows that the saturated subsoil contained approximately 25 per cent of water. By reference to Table I, which shows the percentage of water in saturated soils of various densities, it will be seen that this Huntley subsoil may be assumed to have an average density of 99 pounds of dry soil per cubic foot. Thus, each cubic foot of the saturated subsoil contains 99 pounds of soil and 25 pounds of water. This 25 pounds of water per cubic foot is equivalent in volume to 4.8 inches of water per square foot of area.

In connection with the moisture determinations described above, tests were made of the moisture content of the subsoil just above the level of the ground water. These latter determinations were made on samples representing 6-inch layers, none of which was more than 2 feet above the point of saturation, and most of them were within 1 foot above it. There were in all 80 moisture determinations on the soil just above the water line, and these gave a mean of  $24.8 \pm 0.25$ . This mean when compared with the mean of  $25.4 \pm 0.2$  for the 30 samples reported in Table III shows that there is very little difference in the moisture content of the soil just above and just below the level of the underground water.

These results indicate that it takes very little change in the actual volume of the underground water to cause a marked change in level. It would take only a small contribution of percolating water from above to cause the ground-water level to rise; conversely, the removal of a small volume of water by drainage would lower the ground-water level. The volume relations involved in raising or lowering the ground-water level are probably different with different soil types. Very little informa-

<sup>3</sup> These observations were made at the writer's request by Dan Hansen, Superintendent of the Huntley Experiment Farm.

tion is available concerning these relations. In the case of the soils at Huntley, the figures reported would indicate that a change in water content of 0.6 per cent in the critical zone would change the water level 1 foot.

TABLE III.—*Moisture content of soil saturated with underground water, Huntley, Mont., May, 1923*

Well No.	Depth.	Texture of soil. <sup>a</sup>	Moisture.
			Per cent.
A2.....	12.5	SCC	24.0
	13.0	SSC	25.6
	14.0	SSC	24.8
A4.....	13.5	SSC	25.3
A6.....	11.0	SSC	28.1
A7.....	11.0	SCC	27.4
	11.0	SSC	25.1
B1.....	11.5	SSC	24.6
	10.5	SSC	25.7
B2.....	11.0	SSC	26.0
B3.....	11.0	SSC	23.4
	11.0	SCC	25.6
B4.....	11.5	SCC	26.5
B6.....	10.5	SS	29.6
B7.....	9.5	SSC	25.0
	9.5	SSC	25.0
B8.....	10.0	SSC	24.7
	10.5	SS	22.2
C4.....	11.0	SS	22.7
	10.5	SSC	26.4
C6.....	11.0	SSC	24.6
C7.....	10.0	SSC	25.4
	10.5	SS	23.5
C8.....	11.0	SS	23.0
	10.5	SSC	24.1
C9.....	11.0	SS	23.1
	11.0	SS	26.1
C10.....	11.5	SS	25.6
	10.5	SSC	27.6
C11.....	11.0	SCC	30.8
Mean.....			25.4±0.2

<sup>a</sup> The letters S and C refer to the proportions of sand and clay judged by observation.

An experiment in lowering the ground-water table by pumping, reported from the Salt River Valley, Ariz., (7)<sup>4</sup>, affords a basis for estimating the quantity of water involved in a change of level of underground waters. In this experiment a well was put down 285 feet deep where the water table was 3 feet below the surface. Water was pumped for 630 hours during a period of 34 days, with a total discharge of 77.9 acre-feet. The maximum draw down at the well was 73 feet. The radius of measurable depression of the water table was 2,350 feet, while the radius of pronounced depression was 1,150 feet. It was computed that for the inner area of 95 acres the water table was lowered 4.4 feet and for the outer area of 300 acres it was lowered 0.6 of a foot. The removal of 77.9 acre-feet of water cleared 598 acre-feet of subsoil of its

<sup>4</sup> Reference is made by number (italic) to "Literature cited," p. 692-693.

free water, which is to say that the removal of 1 acre-foot of water changed 7.7 acre-feet of subsoil from a saturated to an unsaturated condition. In other words, the removal of 1.56 acre-inches of water lowered the water table 1 acre-foot. It is probably safe to assume that under the conditions of this experiment there was some inflow of water from the surrounding saturated subsoil that was not observed, so that the ratio 1.56 acre-inches per acre-foot is probably too high rather than too low.

F. H. King (12, *p.* 132-134) has reported on the water content of undisturbed field soils when saturated and when only the bottom inch of each 1-foot section was saturated. From his figures for soil from the third, fourth, and fifth foot sections, there was an average difference of 0.4 of an inch less water in the soil when the bottom only was saturated than when the whole of the section was saturated. In another experiment King set up columns of soil 7 feet long which were first saturated and then allowed to stand for 60 days with the bottom of the soil column in water and the top protected from evaporation. The columns were then cut into 6-inch sections and the water content of each section was determined.

In the results reported by King for this experiment, if the top and bottom 6-inch sections be eliminated, it is found that the water content decreases fairly consistently from the lower to the upper sections. Where the soil used was classed as sandy loam the decrease in water content of each 6-inch section from below upward was at the rate of 0.6 per cent. With the soil classed as clay loam the rate of decrease was slightly less than 0.5 per cent per section.

These figures when converted into inches of water for each 1-foot layer of soil give an average decrease of water of slightly more than 0.1 of an inch for the sandy loam and slightly less than 0.08 of an inch for the clay loam.

It would appear from such evidence as is available that when considering only the layer of subsoil immediately above the level of underground water, the addition of 0.1 of an inch of water would result in raising the ground-water level 1 foot. As a matter of fact, it would not be correct to conclude that the removal of a volume of water equal to 0.1 of an inch in depth would lower the underground water level as much as 1 foot or that the addition of that quantity would raise it 1 foot for the reason that any change in water level would affect the moisture conditions for some distance above the water line.

The extent of the change of moisture conditions of the subsoil consequent upon or necessary to produce a change of 1 foot in the ground-water level would be influenced materially by the texture and density of the soil material. It is probable that in some situations the addition or removal of half an inch of water might raise or lower the ground-water level 1 foot while in other situations it might require  $1\frac{1}{2}$  inches to cause the same change. Speaking generally, it may be assumed that the addition or removal of 1 inch of water may raise or lower the ground-water level 1 foot.

## CONDITIONS OF SOIL WATER

It has been customary to say that water may be contained in the soil in three different conditions which are designated as follows:

- (1) Hygroscopic water, or water that is held by the soil when in equilibrium with the air.
- (2) Capillary water, or water that is held as liquid films around the soil particles in such a way as to exert no hydrostatic pressure; in other words, in equilibrium with the force of gravity, but not in equilibrium with the air.
- (3) Hydrostatic water, or water that exists in the soil, but subject to movement in response to the force of gravity unless such movement is hindered by some barrier.

While this classification is useful in connection with the study of the physical properties of soils, it does not serve so well in dealing with the relations between the soil and the crop plant. The soil may be regarded as a reservoir which is replenished with water from time to time by rainfall or by irrigation, from which there may be losses by percolation or evaporation and from which the plant draws its daily supply. The effective reservoir capacity of the soil is limited on the one hand by

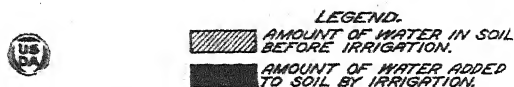
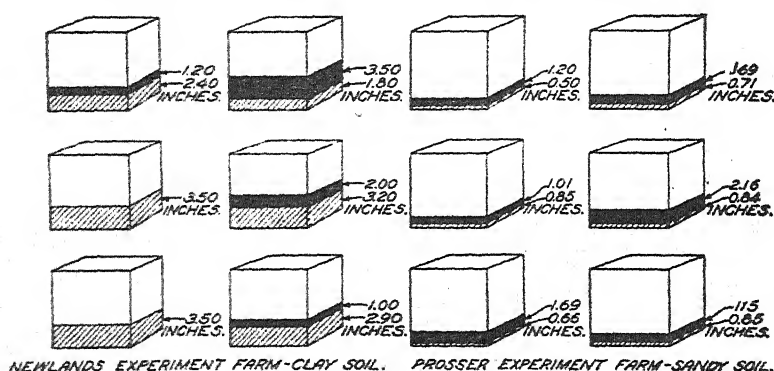


FIG. 2.—Quantity of water held before and after irrigation by two soils.

the fact that most crop plants do not thrive when the soil is saturated with water so that air is excluded and on the other hand by the fact that plants can not absorb all of the water held in the soil.

From the standpoint of its use by crop plants, the soil water may be classified as to condition as follows:

- (1) Subavailable water, or that portion of the soil water which is held by the soil when plants can no longer obtain water for the normal processes of growth. The moisture content of the soil which represents the upper limit of the subavailable supply is often referred to as the wilting point of the soil.
- (2) Available water, or that portion of the soil water that is in excess of the subavailable supply and has an upper limit somewhat below the saturation point.
- (3) Superavailable water, or that portion of the soil water between the saturation limit and the limit above which plant roots do not function normally, presumably because of lack of air.

The soils which one finds in irrigated fields differ so greatly from place to place that it is impossible to indicate in any precise way how much water any soil may hold, even were it possible to establish definitely

the limits of the classes of soil water listed above. The class of water with which the irrigation farmer is chiefly concerned is the one designated available water.

The quantity of this available water that may be added to a soil by an irrigation may be determined by sampling the soil to the depth of the root zone when the crop shows evidence of needing irrigation and sampling again to the same depth as soon as possible after irrigation. The increase in moisture content of the soil shows how much available water has been added by irrigation.

The results of such an investigation with reference to two very different types of soil are shown in Table IV. The figures in the table give, in terms of the percentage of water to the dry weight of the soil, a measure of the increase in the water supply of the 3-foot layer of soil due to an irrigation. These percentage figures may be converted into their equivalents in terms of inches of water for each foot of soil and thus express the volume of water in a more concrete way than the percentage statement does. The volume relations of the soil and the water it contains are shown in figure 2, which is based on Table IV. In converting percentages of water to inches of water it was assumed that the clay soil had a density of 85 pounds per cubic foot and the sandy soil a density of 82 pounds per cubic foot.

TABLE IV.—Quantity of water in the soil to the depth of 3 feet before and after irrigation at two locations on each of two kinds of soil

Depth.	Quantity of water expressed as a percentage of the dry weight of the soil.			
	Newlands Experiment Farm, clay soil.		Prosser Experiment Farm, sandy soil.	
	Location 1.	Location 2.	Location 1.	Location 2.
First foot:				
Before irrigation.....	14.7	11.0	3.2	4.5
After irrigation.....	22.6	32.5	10.8	15.2
Second foot:				
Before irrigation.....	21.4	19.6	5.4	5.3
After irrigation.....	21.4	31.8	11.8	19.0
Third foot:				
Before irrigation.....	21.4	17.7	4.2	5.4
After irrigation.....	21.4	23.8	14.9	12.7

In figure 2 each column of cubes represents a column of soil to the depth of 3 feet, each cube representing 1 foot of soil. The quantity of water in each foot of soil before irrigation is represented by the shaded portion of the cube, and the quantity of water added by irrigation is shown by the black portion. In the first column, on the left, the soil contained before irrigation 9.4 inches of water to the depth of 3 feet, the second column 7.9 inches, the third column 1.91 inches, and the fourth contained 2.4 inches.

The soil represented by column 1 in figure 2 was not in crop when the samples were taken. It was a bare spot in a field of alfalfa where the soil did not take water readily. The irrigation which followed the sampling added only 1.2 inches of water to the soil and that increase was

limited to the surface foot. The soil represented by the second column in the figure was located in the same plat as that shown in column 1 and only 15 feet from it. In this location there was a good stand and a good growth of alfalfa nearly ready to cut. The same irrigation that added 1.2 inches of water to the soil shown in column 1 added 6.5 inches of water to the soil shown in column 2. Both these soils were rich in clay.

The soils of columns 3 and 4 were of a sandy type and were taken from adjacent fields on the Prosser Experiment Farm, Wash. Both fields were carrying a good growth of alfalfa about ready to cut. Irrigation added 3.9 inches of water to the soil shown in column 3 and 5 inches of water to the soil shown in column 4.

It is altogether probable that when these soils were sampled before irrigation the available water supply in the first 3 feet had not been entirely exhausted. But the growth conditions were such that it was deemed advisable to irrigate. It is not always safe nor is it good farming to force the plants to use the last drop of available water.

It is also probable that in the sandy soil of the Prosser Experiment Farm the roots of alfalfa extend below the third foot. Notwithstanding these reservations with respect to the conditions which these figures represent, they afford a comparison as to the relative quantities of sub-available and available water in each location. This comparison is shown in Table V.

The results of a large number of observations covering a wide range of soil types indicate that the capacity of the irrigated soils for holding available water ranges from about 1 inch per foot up to 2 inches per foot, or possibly a little more in exceptional cases.

TABLE V.—Quantities of water held in the first 3 feet of soil in four locations on two different types of soil

Classification of water.	Quantity of water expressed in inches of depth.			
	Newlands Experiment Farm, clay soil.		Prosser Experiment Farm, sandy soil.	
	Location 1.	Location 2.	Location 1.	Location 2.
Subavailable.....	9.4	7.9	1.9	2.4
Available.....	1.2	6.5	3.9	5.0

#### THE PENETRATION OF IRRIGATION WATER

It is the aim in applying irrigation water to moisten the soil to the depth of several feet and in doing so to store enough water in this layer of soil to meet the needs of the crop plants for two or three weeks, or until the next irrigation. If this aim is to be realized, it is essential that the water penetrate the soil readily, for it is often not practicable to keep water on the land more than a few hours at a time.

When irrigation water is applied to the land, it penetrates in part by flowing into such cracks in the soil as have been formed by shrinkage and in part by percolating into the minute spaces between the soil particles. Where the soil conditions are favorable, most crop plants develop their roots to the depth of 4 or 5 feet and are thus able to use the water

that may be stored in that zone of the soil by irrigation. It is often observed that certain irrigated lands do not take water readily and that after the surface of the soil has been saturated for several hours or even for two or three days the water has not penetrated downward into the subsoil. This condition is sometimes thought to be due to the existence of a hardpan or plowsole and that deeper penetration of water could be brought about by deeper plowing or subsoiling. It is possible, of course, to break up a dry soil with a plow, and immediately after this has been done irrigation water will flow downward and fill the spaces between the clods. But very often it is found that by the time of the next irrigation, the soil is nearly as compact and impermeable as it was before it was plowed.

While there are many places in irrigated land where a subsurface hardpan occurs and prevents the downward movement of irrigation water, it is also true that some irrigated soils are naturally slow to take water, and this condition is quite independent of any sharply defined impermeable layer.

It is desirable to understand the facts in these cases, for the remedies are different in the different circumstances. If the penetration of water is retarded or prevented by a well-defined hardpan, such as is sometimes formed by limestone known as caliche, this condition may be remedied by shattering the hard layer by deep plowing or by blasting. On the other hand, if the difficulty is due to what is sometimes termed a "colloidal" or "puddled" condition of the soil, deep plowing or blasting is practically useless. The remedy lies in the direction of improving the physical condition of the soil. One who has not had actual experience with irrigation in districts where so-called "hard soils" or "slick spots" occur does not appreciate how resistant to water such soils are. One thinks of a dry soil as thirsting for water, but on these slick spots the water may stand for days and soak down only a few inches.

#### THE RATE OF WATER PENETRATION

It is possible, though somewhat difficult, to measure the rate at which irrigation water penetrates the soil under field conditions. This may be done by taking a series of soil samples from time to time after the irrigation water is applied. Such investigations have shown that in very permeable sandy soils the water may get down as far as 6 feet in 12 hours. Ordinarily the rate of penetration is much slower. Even in soils that are regarded as readily permeable it may take two or three days for the irrigation water to penetrate to the depth of 6 feet.

The contrast in the rate at which water may penetrate the dry soil may be illustrated by a very simple experiment. If dry pulverized soil is put into a glass tube and water poured on at the upper end, the rate at which the water soaks downward may be observed. The moist soil is much darker colored than the dry soil, so that the advancing line of the penetrating water may be plainly seen and its position may be recorded from time to time by marks on the tube.

The experiment here described illustrates a method of comparing the rate of the penetration of water in two soils, one of which takes water readily while the other takes water slowly. Both soils were taken from the Newlands reclamation project near Fallon, Nev. Both would be classed as sandy loam. When dry and pulverized they resemble each

other so closely as to be almost indistinguishable. In the present experiment these soils were pulverized and passed through a sieve having holes 2 mm. in diameter. The soil was then placed in glass tubes half an inch in diameter and 5 feet in length. The lower end of the tube was fitted with a perforated rubber stopper which was covered with a layer of absorbent cotton. As the soil was poured in, the tube was jarred in such a way as to make the soil settle firmly. The tubes were filled to the 4-foot mark and clamped to a stand in a vertical position. Distilled water was then poured into the top of the tubes to the depth of about 6 inches, and more was added from time to time, as needed. The perforated stoppers in the tubes permitted the escape of air as it was displaced by the descending water. The line of demarcation between the wet and dry soil was very sharp.

Table VI shows the rate of penetration of the water into the soil in the two tubes. In the tube containing soil No. K82 the water penetrated to the depth of 33 inches in three hours, while with soil No. 337 the water had gone down less than 4 inches at the end of the first three hours. In this tube, No. 337, a constant supply of water was kept on the soil for more than a year and the depth of penetration was recorded from time to time. At the expiration of 14 months the water had soaked down to the bottom of the soil column, or 48 inches.

TABLE VI.—*Penetration of water in two samples of dry pulverized soil in glass tubes*

Time elapsed.	Depth of penetration (inches).		
	Soil No. K82.	Soil No. 337.	Rate per day.
1 hour.....	16.7	2.5	.....
2 hours.....	25.0	3.2	.....
3 hours.....	32.7	3.7	.....
4 hours.....		4.1	.....
5 hours.....		4.4	.....
6 hours.....		4.5	.....
1 day.....		5.6	5.6
10 days.....		9.3	.41
30 days.....		12.6	.165
60 days.....		16.6	.133
90 days.....		20.0	.113
120 days.....		23.1	.103
150 days.....		25.5	.080
12 months.....		44.0	.086
14 months.....		48.0	.066

Toward the end of this experiment it became difficult to determine the limit of penetration accurately. Instead of a sharp line of demarcation between the wet soil and the dry, there was a gradual transition of color. Evidently the penetration of water was retarded by conditions in the mass of moist soil in the upper part of the tube rather than by any definitely impermeable area below. It was observed also that the rate of penetration of the water declined rather uniformly as time went on. This has been noted also in other similar experiments.

The outstanding feature of this experiment is that with these two soils, closely similar in physical texture, there is a very great difference



in permeability to water. Such differences are a matter of common observation in the field in irrigated areas and constitute one of the most important distinctions in irrigated soil. The importance of this matter of permeability to water lies in the fact that to function properly in the support of crop plants the soil must act as a reservoir for the storage of water from one irrigation to the next. If the soil is not permeable to water and does not readily absorb water when irrigated, it does not function well in its capacity as a reservoir.

There is another aspect of the case that is also important. Some irrigated soils contain soluble salts, commonly known as alkali, in such quantities that the soil solution is so concentrated as to be toxic to crop plants. When conditions are such that the irrigation water penetrates the soil readily and passes away in the underground drainage, it is not difficult to leach out the excess of soluble salt and thus to reclaim the alkali land. But when the soil is not readily permeable to water, such reclamation becomes difficult or impossible. If conditions are such that the irrigation water soaks down only a foot or two into the soil, it does not carry the salts away. Instead of doing so it evaporates, leaving in the soil not only the salt originally there but also any additional salt which is carried in solution by the irrigation water.

In view of these facts, it is clear that a soil to be used successfully for the production of crops under irrigation must be readily permeable to water, not only that it may serve as a suitable reservoir to hold water for the use of plants but also that any excess of soluble salts may be leached away.

#### MEASURING THE RATE OF WATER PENETRATION

It is not to be supposed that the rate of water penetration in dry pulverized soil in glass tubes in the laboratory is the same as the rate of penetration of irrigation water in the field. There is, however, reason for believing that the differences in the rate of penetration for different soils as shown in laboratory tests are to be found also in the field and that these differences are in the same direction and substantially of the same degree. Numerous comparisons have been made in the laboratory between soils from different fields and different irrigated regions, and these results appear to accord very well with field experience. This field experience shows clearly that the rate of water penetration varies greatly even in different parts of the same field. An example of this variation is shown in figure 2 and in Table IV.

In making the laboratory tests of water penetration as described above there are certain factors that make for a lack of uniformity. Even when portions of the same soil sample are used in different tubes there are likely to be differences in the degree of fineness of the material used and differences in the way the material settles together in the tube. Table VII shows the differences observed in the rate of water penetration in 5 tubes that were filled from the same sample of soil and given the same quantity of water. These results show that the differences in rate of penetration are relatively slight when compared with those shown by different soils even of closely similar textures, as shown in Table VI.

TABLE VII.—Penetration of water in each of five tubes containing soil from the same sample

Time elapsed.	Depth of penetration (inches).					
	Tube 1.	Tube 2.	Tube 3.	Tube 4.	Tube 5.	Mean.
1 hour.....	3.7	4.3	4.2	3.9	4.5	4.1
2 hours.....	4.6	5.1	5.1	4.6	5.4	5.0
3 hours.....	5.0	5.6	5.5	5.0	5.9	5.4
4 hours.....	5.4	6.0	5.9	5.4	6.3	5.8
5 hours.....	5.9	6.6	6.5	5.7	6.8	6.3
24 hours.....	11.6	12.2	11.9	10.2	12.2	11.6

Another example of the differences observed in the rate of water penetration in different soils is shown in figure 3. This figure shows diagrammatically the depth of water penetration in glass tubes containing pulverized dry soil under a fairly constant head of 3 to 4 inches of water. The figures in this diagram show that there are very great differences in the rate of penetration of water in different soils. It is to

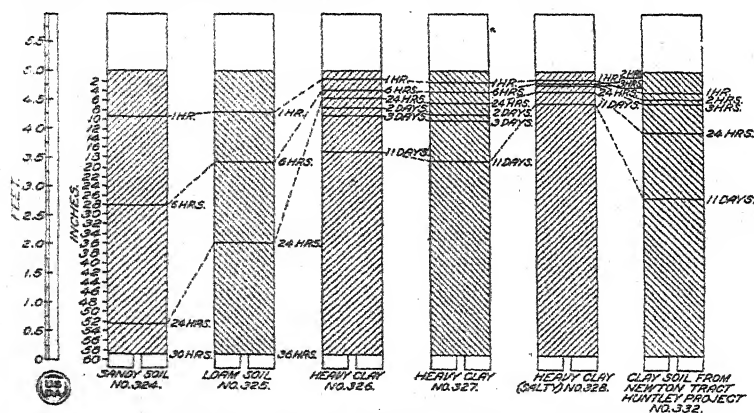


FIG. 3.—Tubes showing rates of moisture penetration in different soils.

be expected that the texture of the soil has an important bearing on the rate of water penetration. This is shown by the more rapid penetration in the sandy soil (No. 324) and the loam soil (No. 325) than in the clay soils of the other tubes.

It is not to be inferred, however, that soil texture is the only factor or that it is even the most important factor influencing the rate of water penetration. It has been shown in Table VI that two sandy loam soils which are indistinguishable as to texture show very great differences in the rate of water penetration. Similar differences are shown in the base of a clay soil from the Belle Fourche reclamation project in South Dakota. This Belle Fourche soil is very rich in clay, having been derived from the weathering of Pierre shale. The four samples shown in Table VIII are all from the same locality and of substantially the same texture. One of them (No. 338) was taken from a spot which had been subjected to the action of seepage water rich in alkaline salts. The other samples were from places that had been watered only by rainfall or irrigated with water in which the proportion of alkaline salts was low as compared with the salts of calcium and magnesium.

It will be seen from the table that the rate of water penetration was six to eight times as fast in the samples that had not been subjected to the action of the alkaline salts as it was in the other sample. The penetration rate on these unaffected clay soils is at first nearly twice as great as that of the sandy loam soil (No. 337) shown in Table VI. Furthermore, the penetration rate was better sustained in the clay soil than in the slowly permeable sandy loam, as is shown by the depth of water penetration in 30 days.

The results of the laboratory experiments with dry pulverized soils in glass tubes afford a means of stating in definite terms what is continually to be observed in the field. They serve to establish two facts which have long been known to irrigation farmers: (1) That some soils absorb water readily while others do not; and (2) that the rate of water absorption is affected by factors other than the texture of the soil.

ABLE VIII.—Penetration of water in each of four samples of heavy clay soil from the Belle Fourche project in South Dakota

Time elapsed.	Depth of penetration (inches).			
	Soil No. 338.	Soil No. 339.	Soil No. 340.	Soil No. 341.
1 hour.....	0.8	4.2	3.4	3.7
6 hours.....	1.5	9.7	7.6	8.2
1 day.....	2.3	17.8	14.3	15.3
2 days.....	2.8	22.2	18.5	19.3
4 days.....	3.5	26.8	22.5	23.2
6 days.....	3.8	30.2	25.8	26.0
10 days.....		34.6	30.1	29.6
30 days.....		42.1	37.4	38.2

### THE PERCOLATION OF IRRIGATION WATER

In the preceding pages reference has been made to the penetration of water into pulverized dry soil. Another situation which has to be dealt with in irrigation practice is the movement of water through a soil already saturated or nearly so. This movement of water through a saturated soil is here designated as percolation. The justification for making a distinction in the present use of these two terms is merely one of convenience. The movement of water into a dry soil may be held to be percolation as truly as the movement of water through a saturated soil. In the first case, the moving water partially or completely displaces air, and in the second case it displaces the water already present.

It is a matter of convenience, however, to distinguish the two conditions by specific terms. In the application of irrigation water to a field the purpose ordinarily is to restock the soil reservoir with water for the use of crop plants. In this process the chief concern is that the water applied to the surface of the field shall enter the soil promptly and be held within the root zone for subsequent use by crop plants.

There are situations which occur not infrequently in irrigated lands where the movement of water through a saturated soil has to be considered as a special problem, and the factors which influence this movement need to be understood. When the water that is held in the soil contains so much dissolved salt as to be injurious to plant growth, it

becomes necessary to displace it by applying fresh water to the surface of the soil. This process, which is known as leaching, is commonly resorted to in the reclamation of saline soils.

The reclamation of saline soils by leaching is possible where the fresh water applied on the surface can percolate readily into the soil and where it is possible for the salty water already present in the soil to move downward into the subsoil or laterally to drainage channels. It is, consequently, a matter of importance to understand the conditions that influence

the percolation of water through the soil in dealing with the problem of reclaiming saline soils.

On the other hand, one of the ways that irrigated soils become saline is through the percolation into them of salt-bearing water. The evaporation of such water from the soil leaves the salt behind to be redissolved in succeeding waters. In order to understand the conditions that influence the rate of movement of percolating waters through the soil, it is necessary to be able to measure the rate of movement. This measurement of the movement of percolating water under field conditions is not less difficult than measuring the rate of the penetration of water into the dry soil.

A simple case of the movement of water through a moist or saturated soil may be described as an example of percolation. Let it be assumed that an irrigated field has an underground water table not

far below the surface and that the field is provided with a drainage system that affords an outlet for the surplus water. When such a field is irrigated the water applied to the surface of the ground soaks downward and displaces some of the water already present in the soil, and the discharge from the drainage system is correspondingly augmented. It is possible to demonstrate that the water discharged into the drainage system has been displaced from the soil and that it is not the water just put on by irrigation.

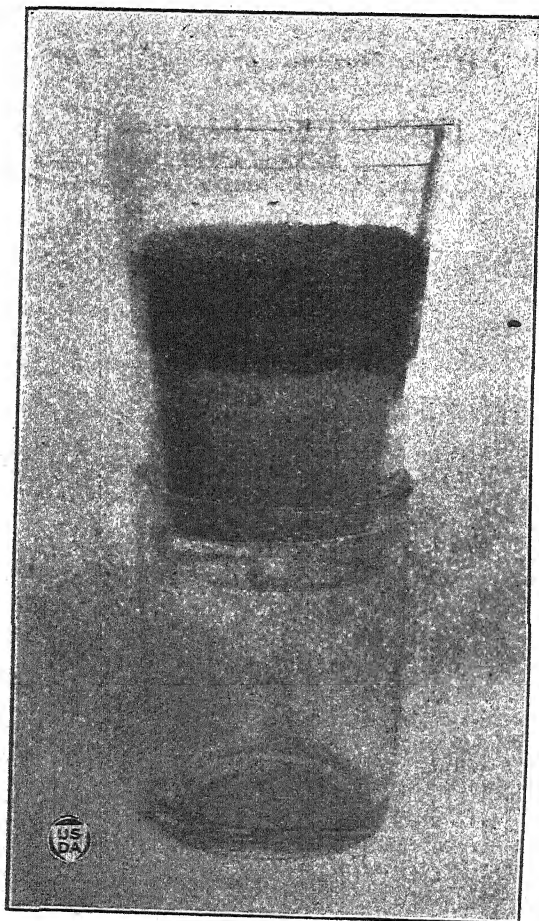


FIG. 4.—Glass used for soil leaching and permeability experiments.

This may be done by means of chemical analyses of the two waters. By this means the quantity and character of the substances dissolved in the water may be determined and the identity of the water may be established.

While it is possible by the means indicated above to demonstrate that the percolation of irrigation water proceeds by displacement, it is not a simple matter to determine the rate of this displacement under field conditions. The rate of displacement differs greatly in different parts of the same field because of differences in the texture or structure of the soil, and there is also some mixture of the water and some diffusion of the dissolved material from each water to the other.

Something as to factors that influence the percolation of water through the soil may be learned in the laboratory by the use of the simple device illustrated in figure 4. This consists of a glass pot with a small hole drilled through the bottom to provide drainage. The pot shown in the figure is  $3\frac{1}{4}$  inches across the top and  $4\frac{1}{2}$  inches high. It will hold conveniently 300 gm. of soil, leaving space for as much as 100 cc. of water at the top. The loss of soil through the drainage hole may be prevented by placing a small filter paper in the bottom of the pot before putting in the soil. The leaching pot may be set into an ordinary drinking glass to collect the percolate and may be covered with a Petri dish to prevent evaporation from the moist soil.

This apparatus is useful not only for measuring the percolation rate of soils but for investigating the changes that take place in the character of the soil solution during the process of leaching. It is possible also with this apparatus to determine the relative water-holding capacities of different soils, though the water-holding capacity shown in this way is usually much higher than that shown by the same soil under field conditions.

The percolation rate in cubic centimeters per minute as determined in the laboratory by means of the apparatus described above using several different types of soil was as follows: Soil sample No. 326, 0.049; No. 338, 0.100; No. 325, 0.273; No. 324, 0.353; No. 340, 0.506; No. K82, 0.560; No. 339, 0.701; No. K90, 2.109.

The rates of percolation given represent the mean of several observations. A quantitative expression of the percolation rate with two different soils is shown in figure 5.

With soil No. 324, which showed a percolation rate of 0.353 cc. per minute, the discharge for 12 hours would be 254 cc. With soil No. 326, having a percolation rate of 0.049 cc. per minute, the discharge in 12 hours would be only 35.6 cc. A comparison of the percolation rates shown by these two soils with the rate of water penetration shown by the same soils in figure 3 would indicate that the factors that influence the movement of water into a dry soil tend also to influence in the same way the movement of water through a saturated soil.

#### FACTORS INFLUENCING THE PERCOLATION RATE

It can not be doubted that such factors as the texture of the soil and its structure<sup>5</sup> have a large part in determining the rate of percolation. In fact, if the meaning of the term "structure" is made broad enough it

<sup>5</sup> As here used the phrase "texture of the soil" refers to the composition of the soil as determined by mechanical analysis or to the sizes of the particles composing it. The word "structure" refers to the arrangement of the soil particles in relation to each other, as in the formation of granules or of larger aggregates and in the development of shrinkage cracks on drying.

might include most of the factors that influence percolation rate. The relationship of soil texture to water percolation is too obvious to require much elaboration. When the soil is composed chiefly of large particles the interstitial spaces are correspondingly large and free movement of water is possible. When the soil is composed largely of small particles, the interstitial spaces are much smaller and the movement of water is consequently retarded. The water relations of the soil may be more easily explained and understood by recognizing three main classes of soil material: (1) Sand, (2) clay, and (3) soluble salts.

The class designated as sand may be taken to include all material of whatever size that is crystalline and nearly or quite insoluble in water. The class designated as clay includes the noncrystalline material, also nearly or quite insoluble in water but differing from the sand in that it is very much more finely divided. The soluble salts are mostly crystallized when dry and dissolved or dissociated when wet. In this classification of the soil material the matter of the size of the particles is not

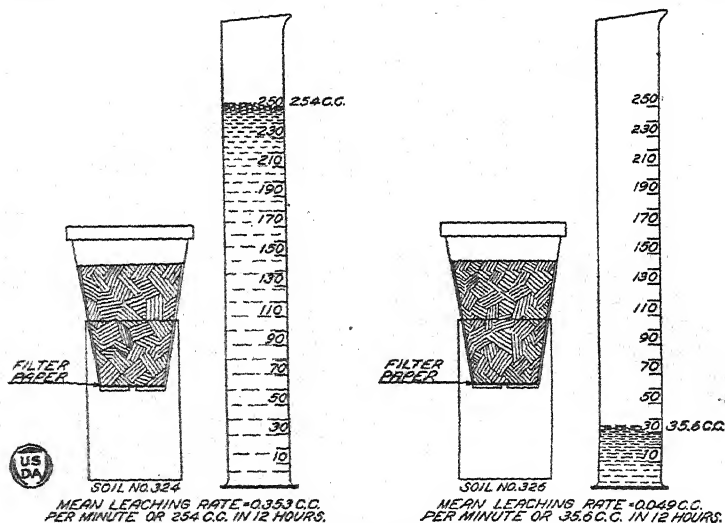


FIG. 5.—Quantity of water leached through different soils in 12 hours.

emphasized. The crystals, particles, or aggregates may be larger or smaller. The distinctions are made on the basis of the reactions with water.

With respect to these reactions with water, the material classed as sand is regarded as nearly or quite inert. The clay is definitely reactive in that though not soluble in water certain of its constituents may enter into reactions with substances dissolved in the water. The soluble salts are more completely reactive than the clay. They pass into solution and dissociate. They move freely with the movement of water in the soil and even move somewhat by diffusion independently of the movement of the water.

Of the three classes of soil material just described, the clay exercises the most important influence on the movement of water through the soil. The extent and character of this influence varies greatly, depending upon the physical properties of the clay rather than upon the quantity in proportion to the total soil mass. The physical properties of the clay are

in turn largely influenced by its chemical composition, and its chemical composition is largely influenced by the character of the salts dissolved in the soil solution.

Thus it is possible to modify very greatly the rate of water movement through the soil under a given set of conditions by changing the character of the salts dissolved in the soil solution. There is abundant evidence bearing on this point which is well known to all investigators of irrigated soils and to many practical irrigators (6, 16).

The crux of the matter appears to be that clay in the presence of water containing dissolved salts participates in chemical reactions by which exchanges may take place between certain elements combined with the clay and other elements dissolved or dissociated in the water. As a result of these exchange reactions the chemical composition of the clay is altered and in consequence its physical properties also. It is to be noted that the changes in the physical properties of the clay appear to be disproportionately large as compared with the changes in chemical composition resulting from these exchange reactions.

The manifestation of the changes that may take place in the physical properties of clay are not confined to its effect on the movement of water through it. It is shown also in the behavior of the soil on drying and in that complex of properties known as plasticity.

From the above observations it seems safe to conclude that the most important factor influencing the rate of percolation in soil is to be found in the clay portion and also that the percolation rate is determined by the physical condition of the clay rather than by its relative quantity in the soil.

#### THE SOIL SOLUTION

It will be apparent from the foregoing statements that the movement of water into or through the soil of an irrigated field is largely influenced by the character of the material in solution in that water. This relationship between the character of the salts in the soil solution and the permeability of the soil is an aspect of the so-called alkali problem that has not been extensively investigated. By far the larger part of the investigations concerning alkali troubles have been concerned with the relationships between the crop plants and the soil solution.

It is fairly well recognized that when the quantity of dissolved material in the soil solution becomes excessive, crop plants are injured. There is no definite agreement as to what proportions of dissolved material in the soil solution are to be regarded as excessive. In fact, it has been so difficult as to be almost impossible to determine what may be called the critical concentrations of salts in the soil solution. Numerous investigations have been made to determine the toxic limit of solutions with plants grown in water cultures, but the translation of these results into terms of the concentration of the soil solution has not been satisfactory.

The direct determination of the quantity or of the character of the material dissolved in the soil solution is rendered difficult by the fact that it has not been practicable to obtain samples of this solution as it exists in the soil under such conditions as make it available to the plant roots. In other words, it is very difficult to extract water from the soil when the water content is no higher than what may be regarded as optimum for plant growth.



In ordinary investigational work where the salt content of the soil solution is the subject, it is customary to treat a sample of the soil with a large quantity of water and then filter off a part of the water for examination. Some investigators use 5 parts by weight of water to 1 part of soil. Others use 10 parts of water and still others use 20 parts of water to 1 part of soil. There are also some differences of methods as to the length of time the water is left in contact with the soil samples and as to the extent to which the mixture of soil and water is agitated.

It is assumed that the quantity of water used is adequate to dissolve all of the readily soluble material in the soil. An examination of an aliquot part of the water used in treating the soil sample is then made, and the result of this examination multiplied by the appropriate factor gives the quantities of the dissolved materials in terms of the dry weight of the soil. Thus the salt content of irrigated soils is usually given in terms of the percentage of the dry soil rather than as a percentage of the soil solution. It is obviously not practicable to attempt to state the salt content in terms of the soil solution, for two reasons: (1) Because the quantity of the soil solution and consequently its concentration changes from day to day as water is lost by evaporation or added by rainfall or irrigation, and (2) because there is reason for believing that the quantity of material in solution is affected by the quantity of water present. It is largely because of this second reason that investigators hesitate to express the results of their soil analyses in terms of concentration of the soil solution, even when the water content of the soil has been determined.

A single example will serve to show the range in concentration of the soil solution if it is assumed that the salt content of the soil is all dissolved in the soil solution. Where the salt content of the soil is 0.5 per cent and the moisture content is 25 per cent it might be assumed that the salt content of the soil solution would be 2 per cent. On the other hand, if the salt content is 0.5 per cent and the moisture content is 10 per cent then the salt content of the soil solution would be 5 per cent.

It is the consensus of opinion among investigators that the roots of ordinary crop plants can not tolerate concentrations of mixed salts in solution much above 1.5 per cent. Notwithstanding this, many instances are reported of crop plants making fair growth in soils which show by analysis as much as 0.5 per cent of salt to the dry weight of the soil and where the moisture content frequently falls as low as 15 per cent.

Such observations have led to the conclusion that when the soil solution becomes very concentrated through the loss of water by evaporation, some of the salt may be reabsorbed in some way by the soil.

#### EXTRACTION OF THE SOIL SOLUTION

While it is difficult to obtain a sample of the soil solution when the water content of the soil is at or near the wilting point, it is possible by the use of a centrifuge to obtain a sample when the water content is up to or just above the optimum for plant growth. The centrifuge used in the experiment here described was one designed by Briggs and McLane for use in determining the moisture equivalent of soils (2). The machine used differed from the one described in the publication cited in that it was equipped with an outer casing which serves to collect the water that is thrown out of the soil during the run.<sup>6</sup>

<sup>6</sup> The writer is indebted to J. W. McLane for his cooperation in making these solution extractions.



In making these solution extractions the dry soil was placed in the centrifuge cups and then carefully moistened to a point just below saturation. It was left in this condition overnight, protected from evaporation, and then centrifuged. The solution extracted represented that part of the soil solution that is above the limit of the moisture equivalent and below the limit of saturation. The moist soil was weighed both before and after centrifuging, and the dry weight was obtained later. From these figures the percentage of moisture in the soil was determined before and after the run, and it was assumed that the sample of the solution extracted represented a moisture condition midway between the two. The salt content of the extracted solution was determined by means of the electrolytic bridge. Table IX shows the salt content of the solution extracted from four samples of soil.

TABLE IX.—Salt content of the soil solutions extracted by the centrifuge process from four samples of soil

Sample No.	Percentage of solution to soil. <sup>a</sup>	Salt content (per cent.).	
		Of solution.	Of soil (indicated).
384.....	33	1. 020	0. 337
385.....	36	. 050	. 018
386.....	33	. 062	. 020
385a <sup>b</sup> .....	30	2. 200	. 660

<sup>a</sup> This is the midway figure between the percentage of moisture in the sample before the run and the percentage after the run.

<sup>b</sup> Sample 385a was a duplicate of 385 to which approximately 0.6 per cent NaCl was added before the solution extract was made.

Another method of extracting a sample of the soil solution has been used in the laboratory by the writer. It has the advantage of not requiring the use of the centrifuge. By this method a portion of the soil solution is obtained by displacement. A leaching pot, such as is shown in figure 4, is used for this purpose. After placing a small filter paper in the pot, a sample of 300 gm. of dry soil is poured in a little at a time and enough distilled water is added with each portion of the soil sample to moisten just below the saturation point. The pot is then covered to prevent loss of water by evaporation and allowed to stand for 24 hours to reach a condition of approximate equilibrium. It is then weighed to determine the moisture content.

The quantity of soil used will usually hold from 90 cc. to 150 cc. of water. It has been found that by adding from 25 cc. to 50 cc. to the surface of the soil in the pot this added water will displace approximately an equal quantity which leaches out from below. A test of successive fractions of the percolate has shown that its salt content remains fairly constant until the larger part of the original soil solution has been displaced.

The salt content of the soil solution obtained by this method of displacement from samples of the same soils used in the centrifuge test is shown in Table X, together with the computed salt content of the soil. The moisture percentage shown in the table is the result obtained by weighing the moist soil just before leaching.

TABLE X.—Salt content of the soil solutions obtained by displacement from four samples of soil

Sample No.	Percentage of solution to soil.	Salt content of solution.	Indicated salt content of soil.
		<i>Per cent.</i>	<i>Per cent.</i>
384.....	55	0.595	0.327
385.....	47	.059	.028
386.....	47	.070	.033
385a.....	46	1.510	.694

The samples of the soil solution obtained either by the centrifuge or by displacement may be considered as representing a condition when the soil contains an ample supply of water for plant growth. A number of investigators have proposed methods of obtaining samples of the soil solution when the moisture content is at or just below what is considered as optimum for plant growth, but none of these methods has as yet come into general use in work with irrigated soils.<sup>7</sup>

In view of the fact that most of the investigational work concerning the salt content of soil is based on results obtained by treating the soil sample with quantities of water that supersaturate it, similar treatments were made on portions of the same samples that were used in the experiments just described.

In each extraction 100 gm. of soil were used. For one set this soil was treated with 100 cc. of water, for the next 250 cc. of water, for the third 500 cc. of water, and for the fourth 1 litre of water. The wetted samples were held for 24 hours and were shaken repeatedly. A part of the water was then filtered off and tested for total salts. The results of these tests together with those made on the centrifuge and displacement extracts are shown in Table XI.

TABLE XI.—Salt content of the solution and the indicated salt content of the soil when the soil solution was variously diluted

Sample No.	Percentage of solution to soil.	Salt content of solution.	Indicated salt content of soil.
		<i>Per cent.</i>	<i>Per cent.</i>
384.....	33	1.020	0.337
	55	.595	.327
	100	.287	.287
	250	.127	.318
	500	.065	.325
	1,000	.030	.300
385.....	36	.050	.018
	47	.059	.028
	100	.038	.038
	250	.016	.040
	500	.011	.055
	1,000	.007	.070

<sup>7</sup> The following papers are among the more recent contributions to the subject of extracting the soil solution and each contains citations of earlier work.

PARKER, F. W.: METHODS OF STUDYING THE CONCENTRATION AND COMPOSITION OF THE SOIL SOLUTION (14). BURGESS, Paul S.: THE SOIL SOLUTION, EXTRACTED BY LIPMAN'S DIRECT PRESSURE METHOD, COMPARED WITH 1% WATER EXTRACTS (5). BURD, John S., and MARTIN, J. C.: WATER DISPLACEMENT OF SOILS AND THE SOIL SOLUTION (4). TULAIKOV, N. M., and KUSMIN, M. S.: ON THE QUESTION OF OBTAINING THE SOIL SOLUTION (19).

TABLE XI.—Salt content of the solution and the indicated salt content of the soil when the soil solution was variously diluted—Continued.

Sample No.	Percentage of solution to soil.	Salt content of solution.	Indicated salt content of soil.
		Per cent.	Per cent.
386.....	33	.062	.020
	47	.070	.033
	100	.035	.035
	250	.019	.048
	500	.012	.061
	1,000	.008	.080
385a.....	30	2.200	.660
	46	1.510	.694
	100	.665	.665
	250	.262	.655
	500	.130	.650
	1,000	.066	.660

The outstanding features of Table XI are that these soils not only differ greatly in their percentages of soluble material but they differ also with respect to the solubility of that material.

In the case of soil No. 384 the indicated salt content is substantially the same when computed from the centrifuge extract as when computed from the extracts made with large quantities of water. The same is true with soil No. 385a.

With the other two samples, the results are different. With them the indicated salt content of the soil is very much higher when an excess of water is used than when the solution is nearer the normal of field conditions.

These examples are given here to show why it is that investigators hesitate to estimate the concentration of the soil solution from the results they obtain by testing dilute water extracts of the soil.

#### COMPOSITION OF THE SOIL SOLUTION

Most investigators who are working with solutions obtained from irrigated soils do not attempt to identify all of the substances contained in these solutions. Many of the dissolved materials occur in very minute quantities, and it is probable that they do not have much effect either on the soil or on the plants. There is a wide diversity in the methods used by different workers in the examinations of soil solutions, a term which is here used to include not only the water extracts from soil samples but irrigation and drainage waters as well.

Even in estimating the total quantity of dissolved material there are differences of method. A few of the methods in general use may be listed and briefly described as follows:

(1) A measured quantity of the solution is evaporated to dryness over a steam bath and then dried to constant weight in an oven at a temperature slightly above the boiling point of water; the dried residue is weighed and reported as total dissolved solids in terms of percentage of the original solution or in parts per million.

(2) The same as No. 1 except that the residue after being weighed is heated to low redness to volatilize the organic matter and then weighed again. The loss in weight from heating is then reported as organic matter, and the final residue is reported as total salts.

(3) The electrical conductivity of the solution is measured by the use of a Wheatstone bridge. The bridge reading may be converted in terms of percentage salt content by the use of a calibration table.

(4) A fairly complete analysis may be made, determining quantitatively each of the constituents found to be present and the sum of these reported as the total salts.

(5) A few of the more important acids may be determined volumetrically and these results calculated to their equivalents as salts of sodium, added together, and reported as total salts. To do so involves assumptions that may be misleading. All that is known concerning the composition may be given quite as clearly by reporting the quantities of the various elements or ions that have been identified.

The various constituents of the soil solution are usually reported as parts per million. It is convenient to remember that parts per million may be converted into parts per hundred or percentage by moving the decimal point four places to the left, e. g., 12,000 parts per million equals 1.2 per cent. Likewise, where there is occasion to convert parts per million into pounds per acre-foot of water or of soil, it may be remembered that an acre-foot of water weighs approximately 2.75 million pounds and an acre-foot of soil weighs about 4 million pounds. Thus, if a sample of water is found to carry 730 parts per million of salt that would be equivalent to 1 ton of salt per acre-foot of water.

There are in addition a number of modifications of these methods, but these examples serve to show something of the diversity of methods that are in general use for estimating the salt content of the soil solution. The material dissolved in the soil solution is assumed to exist as salts in equilibrium as to acids and bases. The methods of chemical analysis do not permit the identification of these salts as such, but only of the acid or basic radical or ion. Thus, we may determine with a fair degree of accuracy the quantity of chlorin (Cl), of sulphate ( $\text{SO}_4$ ), or nitrate ( $\text{NO}_3$ ) in a solution and also the calcium (Ca), the magnesium (Mg), or even the sodium (Na), but we can not with certainty know how these exist in the solution with reference to each other. It is questionable whether it is desirable to attempt to state the composition of soil solution or of irrigation and drainage waters in terms of combined salts.

#### IMPORTANT CONSTITUENTS OF THE SOIL SOLUTION

The term soil solution as here used is intended to include not only the solution as it exists in the soil of an irrigated field but also the accumulated underground water or drainage and the water used for irrigation. In other words, water that has been in contact with soil is here termed soil solution. It is not intended to include as a part of the soil solution any of the inert suspended matter that may be removed by careful filtering.

The more important constituents of the soil solution as it exists in relation to irrigated soils may be enumerated as follows: The bases are calcium, magnesium, sodium, and potassium. The acids are sulphate ( $\text{SO}_4$ ), chlorin (Cl), bicarbonate ( $\text{HCO}_3$ ), carbonate ( $\text{CO}_3$ ), nitrate ( $\text{NO}_3$ ), phosphate ( $\text{PO}_4$ ), and silica ( $\text{SiO}_2$ ). Other bases, such as manganese, iron, and aluminum, are sometimes found in soil solutions, and some other acids, particularly organic acids, also occur, but these are not often separately identified.

There is some diversity in the methods used for quantitative determination of the constituents of the soil solution. It is not the purpose here to give a detailed description of these methods, but merely to give an account of them that may serve as a basis for a later discussion of soil reactions in which the character of the soil solution, as determined by these methods, plays an important part.

## CALCIUM

The fact that calcium forms an insoluble compound when combined with oxalic acid is the basis of one group of calcium determinations. The precipitated calcium oxalate may be separated by filtering and the quantity of calcium weighed as the oxid after burning or the quantity of combined oxalic acid determined volumetrically by the use of potassium permanganate.

Another reaction of calcium is also used to some extent. This is its reaction with soap. Both calcium and magnesium form insoluble compounds with soap, and consequently it is possible to estimate the quantity of calcium and magnesium there is in a solution by using a standard soap solution added a little at a time. The solution being tested is shaken in a bottle after each addition of the soap solution. When all the calcium and magnesium have been precipitated by the soap, the solution quickly forms a lather when shaken.

## MAGNESIUM

The gravimetric determination of magnesium is based on the fact that this base forms an insoluble precipitate with phosphoric acid. When all the calcium has been precipitated from a solution by the use of oxalic acid, as mentioned above, the addition of sodium phosphate results in the formation of magnesium phosphate which may be filtered off and weighed.

The magnesium content of a solution may be estimated by first determining the total content of calcium and magnesium, as with the soap solution; the calcium content may then be determined on another sample of the solution and the magnesium estimated by difference.

## SODIUM AND POTASSIUM

The accurate gravimetric determination of sodium and potassium in solution is rendered difficult by the fact that most of the compounds of these bases are soluble. The usual method of procedure is to separate out the other bases and also remove all the sulphate from the solution, replacing it with chlorin to combine with the sodium and potassium and then evaporate to dryness and weigh the residue as the chlorids of the two bases. If it is desired to determine the potassium separately from the sodium the residue is redissolved and the potassium is precipitated with platinic chlorid. Many water analysts do not undertake to make direct determinations of sodium and potassium because the processes are difficult and tedious. Having determined the total salts or the totals of the acids and of the calcium and magnesium in the solution they estimate the sodium by difference or by the requirement of the acids after eliminating the quantity of the acids required to combine with the calcium and magnesium.

## SULPHATE

The sulphate content of the solution may be determined by the addition to it of an excess of barium chlorid. This results in the formation of the insoluble barium sulphate which may be filtered off and weighed. The freshly precipitated barium sulphate forms a white turbidity in the solution, so that a fair estimate of the quantity of sulphate may be made by comparing this turbidity with known standards.

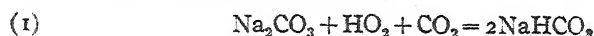
## CHLORIN

Chlorin is determined by titration with a standard solution of silver nitrate, using potassium chromate as an indicator. The first reaction is the formation of insoluble silver chlorid. When all the chlorin has been combined the silver reacts with the chromate to produce a change of color.

## CARBONATE AND BICARBONATE

The conventional method for the determination of these weak acids is to titrate the solution with a standard solution of a stronger acid, such as sulphuric, using phenolphthalein as the indicator for carbonate and methyl orange as the indicator for bicarbonate. The reaction involved in these titrations and the interpretation of their results call for a more detailed statement than has been made concerning the other constituents of the soil solution. This may be justified in part because the carbonates are generally regarded as the most troublesome substances in the solution, both in relation to the soil and the plants, and partly because of some uncertainty as to the significance of the results of the titrations.

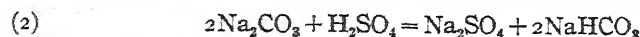
In the first place, it should be kept in mind that the carbonate-bicarbonate complex is very unstable. That is to say, a change in the quantity of carbon dioxide in the solution immediately causes a change in the proportion of carbonate to bicarbonate. This change may be illustrated by the following equation:



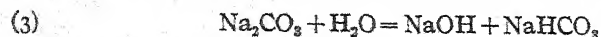
This equation indicates the reaction that takes place when carbon dioxide is added to a solution of sodium carbonate in water, which reaction is in the direction of converting the carbonate to the bicarbonate. The removal of carbon dioxide from the solution, which may be done by aeration or boiling, causes the reaction to take place in the other direction or to change the bicarbonate into the carbonate. The quantity of carbon dioxide in solution is extremely variable, depending upon conditions which are constantly changing, so that there is comparatively little significance to be attached to the distinction between carbonates and bicarbonates in irrigation and drainage waters.

In making a titration for carbonate in a solution, a few drops of phenolphthalein is first added. If the solution takes on a pink or rose color this is assumed to indicate the presence of the normal carbonate ( $\text{CO}_3$ ), and a solution of standard sulphuric acid is added until the color disappears.

The course of the reaction may be illustrated as follows:

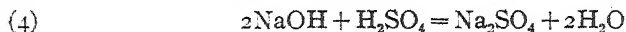


This might be taken to indicate that the pink color of the phenolphthalein indicates the presence of the carbonate radical, but there is another explanation that appears to serve better. Sodium carbonate is an example of a strong base united with a weak acid. When this salt is dissolved in water it is partially dissociated and hydrolyzed as follows:



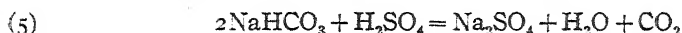
It is not assumed that this reaction is complete, but rather that in a solution of sodium carbonate there exists a certain quantity of free hydroxyl ions ( $\text{OH}$ ) and that these cause the phenolphthalein to show pink

color. According to this explanation the reactions of the carbonate titration would be indicated thus:



It is assumed that only a part of the sodium carbonate is dissociated and hydrolized in the original solution, but that as titration proceeds these reactions go on until the supply of the dissolved carbonate is exhausted. The acid used in titrating the solution until the phenolphthalein color disappears accounts for only one half the original quantity of carbonate. According to the equation (2), the first step of the titration reaction is to form sodium sulphate and sodium bicarbonate. In making carbonate titration the burette reading for the phenolphthalein reaction is doubled before computing the carbonate content of the solution.

Upon completing the titration with phenolphthalein, a few drops of methyl orange is added to the solution and the addition of acid continued until the solution changes color from lemon yellow to orange. By this titration the total quantity of bicarbonate in the solution is determined. The reaction may be indicated as follows:



As has been indicated above, the bicarbonate content of the original solution has been increased when carbonate was present through the conversion of the carbonate to the bicarbonate. Thus in computing the carbonate and bicarbonate equivalents of the acid used in titrating a solution it is necessary to deduct from the burette reading for the methyl orange titration the equivalent of the burette reading for the phenolphthalein titration. The remainder may be taken as the acid equivalent of the bicarbonate originally in the solution.

The correct interpretation of the results of the titrations for carbonates and bicarbonates in soil solutions is beset with still other complications. One of these has to do with the fact that the silicate radical ( $\text{SiO}_3$ ) when it occurs in solution gives the same color reaction with phenolphthalein as the carbonate. This may lead to some confusion in dealing with certain strongly alkaline solutions. Not only does it give too high values for carbonates but it upsets the determination of bicarbonate also. From what has been said above concerning the titration for carbonate it will be evident that if a solution contained carbonate but no bicarbonate its complete titration would require equal quantities of acid to neutralize the phenolphthalein and the methyl orange. With a solution containing silicate this is not the case. It takes much more acid to neutralize the phenolphthalein color than it does to complete the titration with methyl orange. It sometimes happens in laboratory work that solutions are obtained that show an abnormally high proportion of carbonate as compared with the bicarbonate. With such solutions it is advisable to test for silica.

Another source of error in determining the carbonate-bicarbonate constituents of solutions has to do with the evolution or escape of carbon dioxide between the time the sample is taken and the time the titration is made. On one hand, if the solution contains organic matter either suspended or dissolved this may be broken down with the consequent liberation of carbon dioxide. If the solution is stored in a closed container this carbon dioxide may combine with the normal carbonate to form

bicarbonate. On the other hand, it has been observed that certain samples of underground water when first taken show no phenolphthalein reaction, but if these are allowed to stand for a few hours exposed to the air they give a strong reaction for normal carbonate.

One of the practical difficulties in titrating soil solutions is encountered when the solution is strongly colored or turbid, due to the presence of dissolved or suspended organic matter. With such solutions the end point of the indicator reaction is not easy to see. It has been found that this difficulty may be overcome by placing the solution to be titrated in a bottle which can be corked and shaken after each addition of acid. The shaking causes the formation of foam which persists at least momentarily after the shaking stops. The indicator colors can be seen in this foam quite as sharply as in a clear solution.<sup>5</sup>

The discussion of this subject of the determination of the carbonate-bicarbonate constituents of the soil solution may be summarized by pointing out that these determinations are at least difficult to interpret. It is regrettable that this is so because these constituents are known to be closely associated with some of the most important reactions that take place in irrigated soils. They are also regarded as highly toxic to crop plants.

#### NITRATE

The nitrate content of the solution is determined by the use of phenol-disulphonic acid with which the nitrate reacts to produce a yellow color when the solution is made alkaline, as with ammonia. A comparison of the unknown solution with solutions of known strength affords a basis for estimating the nitrate content.

#### SILICA

Silica in solution may be precipitated by evaporating to dryness, acidulating with hydrochloric acid, evaporating again, and finally taking up the soluble residue with dilute acid. The silica may be separated from the other constituents of the solution because it does not redissolve in an acid solution after being dried.

In making a silica determination with soil solutions it is essential to free the solution of all suspended matter. In the water extracts of irrigated soils and in some turbid irrigation waters silica is an important constituent of the suspended matter. In this condition it is not, however, a part of the true solution.

#### STATING THE RESULTS OF ANALYSES

From what has been said on the preceding pages it is obvious that in reporting the results of analyses of samples of the soil solution or of irrigation or drainage waters one can not with certainty report the salts as such. The analysis permits the identification of certain elements or ions, but gives no clue as to how these are combined if at all. Indeed such evidence as we have would indicate that in the dilutions with which we commonly have to deal, these constituents exist largely in a dissociated condition. \*

<sup>5</sup>The writer is indebted to J. F. Breazeale for this detail of technique.



It is doubtful if any useful purpose is served by reporting such analyses in terms of combined salts. The character of the solution may be indicated quite as clearly by reporting the quantities of the constituents identified as by reporting the theoretical combinations which are conjectured. Furthermore, where the report includes only the elements or ions that have been identified it is less subject to misinterpretation than when it is given in terms of combined salts of which only one element or radical has been determined. The results of the analyses of certain underground waters are given in Table XII stated in parts per million. The figures in this table give all the facts that were actually determined in making the analyses.

TABLE XII.—Composition of certain underground waters

Sample No. <sup>a</sup>	Total Solids.	Constituents (parts per million).					
		Ca.	Mg.	HCO <sub>3</sub> .	Cl.	SO <sub>4</sub> .	NO <sub>3</sub> .
1.....	1,614	134	24	249	642	211	.....
2.....	1,453	203	28	163	501	259	19
3.....	534	108	16	236	115	127	.....
4.....	3,570	80	172	<sup>b</sup> 279	312	1,920	.....
5.....	0,142	555	146	504	140	5,896	.....
6.....	5,400	408	543	549	160	2,700	.....
7.....	5,864	270	191	432	84	3,427	.....

<sup>a</sup> The following is a description of the water samples:

No. 1. From wells tapping the underflow of the Gila River above Yuma, Ariz.

No. 2. From wells used for irrigation on the south side of the Salt River Valley, Ariz.

No. 3. From wells used for irrigation on the west side of the Salt River Valley, Ariz.

No. 4. From a drain on the Newlands project, Nevada.

No. 5. From a drain on the Shoshone project, Wyoming.

No. 6. From a drain on the Belle Fourche project, South Dakota.

No. 7. From a drain on the Huntley project, Montana.

<sup>b</sup> Includes 48 parts per million normal carbonate. The other samples showed no reaction with phenolphthalein.

Where it is desired to make a comparison between several different samples of water as to the character of their dissolved constituents such comparison is greatly facilitated by stating the constituent in terms of percentage of the total solids. In order not to have too many items for comparison, constituents having similar properties may be combined in the percentage statement. Thus the calcium and magnesium may be taken together and the carbonate and bicarbonate. In view of the fact that the bicarbonate radical (HCO<sub>3</sub>) is monovalent while the carbonate radical (CO<sub>3</sub>) is bivalent it is customary in combining them in the percentage statement to divide the figure for the bicarbonate by 2 before making the addition.

The combining weights of the bases and acids ordinarily found in irrigation and underground waters are such that the sum of the acids constitutes about two-thirds of the weight of the total solids. As a check on the accuracy of the analytical report, the percentage statement of the results may properly include an item for the total acids. If the figure for this item falls much above 70 or much below 60 the implication is that there has been an error in the work or that some other acid, possibly the nitrate radical, should be looked for.

When the analysis of a water sample includes all the important bases, that is, the sodium and potassium as well as the calcium and magnesium,

the sum of these basic constituents makes up about one-third of the weight of the total solids. Where only the calcium and magnesium have been determined by analysis, the percentage statement of the results indicates approximately at least whether the water is to be classed as "hard" or "soft" with reference to the balance between the two classes of bases.

If the sum of the calcium and magnesium is about 15 per cent of the total solids, it may be assumed that the calcium-sodium ratio is not far from 50-50.

For a discussion of the significance of the calcium-sodium ratio in irrigation waters see Scofield and Headley (16).

An example of a report on the quality of certain underground waters is shown in Table XIII. In this table the figures in the column headed "total solids" give the percentage of these to the original solution. The other figures in the table report the constituents that were identified in terms of percentage of the total solids. With this form of report it is less difficult to compare different waters as to the proportion of their important dissolved constituents even though the total quantities of these constituents are very different. It is obvious that the proportion of calcium and magnesium to the sodium must be low in samples 1, 4, 5, and 7. Sample 3 is high in bicarbonate though low in total solids. Samples 1 and 2 are high in chlorids, while samples 4 to 7 are high in sulphates.

TABLE XIII.—Composition of certain underground waters in which the total solids are stated as percentage of the solution and the important groups of constituents are stated in terms of percentage of the total solids

Sample No.	Total solids.	Constituents as percentage of total solids.					
		Ca+Mg.	CO <sub>3</sub> + HCO <sub>3</sub> z.	Cl.	SO <sub>4</sub> .	NO <sub>3</sub> .	Total acids.
1.....	0.161	9.8	7.8	39.8	13.1	.....	60.7
2.....	.145	16.1	6.1	34.8	17.4	1.3	59.6
3.....	.053	23.2	22.1	21.5	23.8	.....	67.4
4.....	.357	7.1	3.91	8.7	53.8	.....	66.4
5.....	.914	7.7	2.8	1.5	64.5	.....	65.2
6.....	.540	17.6	5.1	3.0	50.0	.....	58.1
7.....	.586	7.9	3.7	1.4	58.4	.....	63.0

The method of reporting the constituents as percentages of the total dissolved solids or total salts does not permit close or accurate comparisons between different waters because these constituents have different molecular or combining weights. For example, 12 parts per million of magnesium has the same combining value as 20 parts per million of calcium. In the same way with the acids, 48 parts per million of sulphate has the same combining value as 35.5 parts per million of chlorin or 30 parts of carbonate. These differences in molecular or combining weight make it impossible to express the true character of solutions by the percentage method of statement. This method is simple and convenient, but at best it permits only approximate comparisons. Where it is desired to make more accurate comparisons between different waters it is advisable to use a method which takes into account these differences

in the combining or reacting values of the constituents. Such a method has been proposed by Stabler (17, 18) and elaborated by Palmer (13) and has been extensively used by water analysts in connection with industrial problems though it has not as yet been used generally in connection with irrigation and drainage investigations. This method of interpreting water analyses is based on the use of what is known as the reaction coefficient for which the symbol *r* is used. The reaction coefficient of an element or ion is determined by the ratio  $\frac{\text{valence}}{\text{atomic weight}}$ . These coefficients for the more important radicals found in irrigation waters are given in Table XIV.

TABLE XIV.—Reaction coefficients for the more important radicals found in irrigation waters

Positive radicals.	Reaction coefficients.	Negative radicals.	Reaction coefficients.
Calcium (Ca).....	0.0499	Carbonate (CO <sub>3</sub> ).....	0.0333
Magnesium (Mg).....	.0822	Bicarbonate (HCO <sub>3</sub> ).....	.0164
Sodium (Na).....	.0435	Chlorin (Cl).....	.0282
Potassium (K).....	.0256	Sulphate (SO <sub>4</sub> ).....	.0208
		Nitrate (NO <sub>3</sub> ).....	.0161

The reacting values of the dissolved constituents are determined by multiplying the parts per million by the reaction coefficient and the results obtained are designated by prefixing the symbol *r* to the symbol for the radical. Thus *r* Ca is the designation for the reacting value of calcium. The application of the method of reacting values to the statement of water analyses is illustrated in Table XV in which it is used with the results given in Table XII.

The reacting values of the various constituents as given in the table are stated in terms of molecular equivalent units. If the analyses were complete and correct the sum of the reacting values of the bases or positive radicals should equal the sum of the acids or negative radicals. In ordinary analytical work the alkaline bases, sodium and potassium, are not usually determined. In the absence of this determination it is not possible to compute directly the proportions of the earthy bases, calcium and magnesium, to the alkaline bases or the percentage of the earthy bases to the total bases. This is a relationship that appears to be important in view of the known differences in the reactions of the elements of these two groups.

In order to make an estimate of the percentage of the earthy bases to the total bases in the solution as well as to be able to make direct comparisons between different waters it is convenient to compute the reacting values of the constituents into percentages. If it may be assumed that the determinations of the acid constituents are approximately accurate, the sum of the reacting values of the acids may be used as a basis for computing the percentages of the constituents. The results of such computation are given in Table XVI. It is believed that this method of statement of the results of analyses gives a fairly clear picture of the essential features of the solution and also permits a fair comparison of one solution with another.

TABLE XV.—*Reacting values of the constituents of certain underground waters, the composition of which is given in Table XII in parts per million*

Sample No.	r Ca.	r Mg.	r HCO <sub>3</sub> .	r Cl.	r SO <sub>4</sub> .	r NO <sub>3</sub> .	r Acids.
1.....	6.69	1.97	4.08	18.10	4.39	.....	26.57
2.....	10.13	3.30	2.67	14.13	5.30	0.31	22.41
3.....	5.39	1.31	3.87	3.24	2.64	.....	9.75
4.....	3.99	14.13	<sup>a</sup> 4.57	8.79	39.95	.....	53.31
5.....	27.70	12.00	8.26	3.95	122.50	.....	134.71
6.....	20.35	44.60	9.00	4.51	56.20	.....	69.71
7.....	13.47	15.70	7.08	2.26	71.15	.....	80.49

<sup>a</sup> Includes 1.6 r CO<sub>2</sub>.TABLE XVI.—*Composition of certain underground waters stated as percentages of the sum of the reacting values of the acids from Table XV*

Sample No.	Total solids.	Percentage of reacting value.					
		r Ca.	r Mg.	r Ca + r Mg.	r HCO <sub>3</sub> .	r Cl.	r SO <sub>4</sub> .
1.....	0.161	25.2	7.4	32.6	15.3	68.2	16.5
2.....	.145	45.2	10.3	55.5	11.9	63.1	23.6
3.....	.053	55.3	13.3	68.6	39.7	33.2	27.1
4.....	.357	7.5	26.5	34.0	<sup>a</sup> 8.6	10.5	74.9
5.....	.914	20.6	8.9	29.5	6.1	2.9	91.0
6.....	.540	29.2	64.0	93.2	12.9	6.5	80.6
7.....	.586	16.7	19.5	36.2	8.8	2.8	88.4

<sup>a</sup> Includes 3.0 per cent r CO<sub>2</sub>.

## THE SOLUBILITY OF SOIL CONSTITUENTS

In dealing with irrigated soils which often contain appreciable quantities of material that is readily soluble in water it is possible to recognize four classes of substances, as follows:

(1) Soluble material, which may be defined as material that is soluble to an extent greater than can be satisfied by the quantity of water in the soil, e. g., the salts of the strong acids with the four principal basic elements and also the carbonates and bicarbonates of the alkali bases. A possible exception in this series is calcium sulphate.

(2) Slightly soluble material, which may be defined as material that is so little soluble that the soil solution is ordinarily saturated with respect to it, e. g., the carbonates of the earthy bases, calcium, and magnesium, and sometimes calcium sulphate.

(3) Replaceable material, which includes the bases, both earthy and alkaline, that are combined with the soil material in such a way that they may pass into solution only if the solution contains an excess of some other base with which the combined bases may exchange, e. g., a soil may be rich in combined calcium and may yield only a trace of this element to treatment to pure water, but when treated with a solution of sodium chlorid it may yield an abundance of calcium.

(4) Insoluble material, which includes the great bulk of the soil. This consists largely of silica either as pure quartz or more commonly as double silicates or compounds of alumina and silica together with one or another of the common earthy or alkaline bases. Some of the double or complex silicates also contain iron and may contain more than one of the bases.

This classification of the soil material, while admittedly an arbitrary one, is useful in that it makes it possible to arrive at a better understanding of the changes that occur in the soil solution as a result of irrigation and leaching and of the reactions that take place between the solution and the

soil. Under field conditions the soil solution is never at rest or in a condition of equilibrium. During the time that irrigation water is being applied the solution is being diluted and is moving downward through the soil. For the remainder of the time it is being concentrated through the loss of water by evaporation and transpiration, and there may be a slight upward movement to replace these losses. In its movement the solution carries its dissolved substances with it. The movement of these dissolved substances by diffusion is probably so slight as to be negligible. For all practical purposes the movement of the dissolved salts in the soil is limited to and determined by the movement of the soil water.<sup>2</sup>

A solution constituent may be precipitated and its further movement arrested in either of two ways. The solution may become concentrated by evaporation to the point of saturation with respect to that constituent, or the constituent, if basic, may take part in an exchange reaction and be withdrawn from solution even though the solution is well below the saturation point with respect to it. It has not been demonstrated definitely that the acid constituents of the soil solution take part in exchange reactions.

There are very pronounced differences in the solubility of the various important constituents of the solution as it exists in irrigated soil. Very little is known as to what these limits are—that is, as to the concentrations at which the precipitation of any given salt takes place in the soil. The precipitation of a salt from solution or, in other words, the limit of solubility may be influenced profoundly by either of two factors. One of these is temperature and the other is the character of the other constituents in solution. The limits of solubility in pure water of the more important constituents of the soil solution in irrigated land are shown in Table XVII. The figures in this table give the limits of solubility in terms of percentage of the water of the solution and also in terms of the reacting values of the elements or ions. These figures show that with most of the salts the limit of solubility increases with the temperature.

TABLE XVII.—*Solubility in water of the earthy and alkaline bases in the presence of the various acids commonly found in the soil solution, expressed as percentage of the solvent and as reacting values<sup>a</sup>*

	Temperature.	Calcium Ca''.		Magnesium Mg''.		Sodium Na'.		Potassium K'.	
		Per cent.	Reacting value.	Per cent.	Reacting value.	Per cent.	Reacting value.	Per cent.	Reacting value.
Carbonate, CO <sub>3</sub> ''.	Cold...	0.0013	0.26	0.0106	2.5	7.1	1,340	89.4	12,900
	Hot...	.088	17.6	.....	.....	45.4	8,570	156.0	22,600
Bicarbonate, HCO <sub>3</sub> '	Cold...	.1	20.0	2.21	520	6.9	821	22.4	2,240
	Hot...	.....	.....	.....	.....	16.4	1,955	60.0	6,000
Sulphate, SO <sub>4</sub> ''....	Cold...	.179	26.3	26.9	4,450	4.8	656	8.5	975
	Hot...	.178	26.2	73.8	12,200	42.5	5,900	26.2	3,000
Chlorin, Cl'.....	Cold...	59.5	10,725	52.2	10,950	35.7	6,100	28.5	3,825
	Hot...	154	28,000	65.9	13,850	39	6,660	56.6	7,580
Nitrate, NO <sub>3</sub> '.....	Cold...	93.1	11,356	200.0	15,620	72.9	8,580	13.3	1,315
	Hot...	351.2	42,829	.....	.....	180.0	21,180	247.0	24,400

<sup>a</sup> From Van Norstrand's CHEMICAL ANNUAL, 1913 (20).

<sup>2</sup> The movement of dissolved salts here referred to should not be confused with the movement of ions in the solution which may take place in connection with the absorption by plants. See Breazeale, J. F. (1)

Our information as to the effect on the solubility of one salt of the presence in the solution of another constituent is far from complete. It is well known that carbon dioxide dissolved in water increases the solubility of calcium carbonate except when the solution contains sodium in excess of the strong acids. Likewise it is known that chlorine in solution increases the solubility of calcium sulphate unless the calcium in solution exceeds the equivalent of the carbonate and sulphate ions in the solution, otherwise the solubility of calcium sulphate is decreased. These examples are cited merely to show that in a system as complex as the soil solution it is not possible to state definitely the limits of solubility. Not only is the system complex with respect to its composition but it is never in equilibrium. It is either in the way of being made more dilute by irrigation or rainfall or of being concentrated by evaporation and withal it is participating in those reactions that are continually taking place between the substances in solution and those combined in the soil.

A consideration of the factors influencing the solubility of the soil material can not well be confined to a discussion of the reactions of inorganic acids and bases. The soil of a cultivated field is teeming with organisms including fungi, bacteria, and nematodes, to name only a few of the groups. It contains also the organic residues of plants and of animals in various stages of decomposition. These organisms and the products of the decomposition of the organic matter have a most important bearing on the character of the soil solution in its relation to crop growth. They doubtless have, also, a bearing on the physical condition of the soil and consequently on its permeability to water. But it is not within the scope of the present paper to deal with these organisms or with the subject of organic matter in relation to the condition or productivity of the soil.

There remains to be considered in some detail one other constituent of the soil and of the soil solution which is believed to play a most important rôle in the matter of the movement of water. This is silica. Although silica is the most abundant substance in the soil, we have as yet very little knowledge of it and of the nature of its reactions. Silica, or the oxide of silicon ( $\text{SiO}_2$ ), was first identified by Berzelius in 1823, though its molecular weight was not established definitely until many years later. It occurs in nature in a great variety of forms, sometimes pure, as in quartz or rock crystal, sometimes combined with water, as silicic acid, but more commonly in combination with one or more of the earthy or alkaline bases. It is the most universal cementing material known.

Notwithstanding the fact that silica is not ordinarily recognized as a constituent of the soil solution, it appears to be unquestionable that it exists in and is often abundant in the solution. The fact that it constitutes an important part of the mineral matter of certain plants and that it is utilized by a great variety of small organisms, such as the infusoria in forming their shells or skeletons, justifies the belief that it exists in a soluble form. Furthermore, it is sometimes identified as an important dissolved constituent of the waters of mineral springs and deep wells. Some water analysts hold the view that the silica found in water is to be regarded as suspended matter rather than as a true solute; in other words, that it should be reported as  $\text{SiO}_2$  rather than as an acid ion  $\text{SiO}_3$ . Yet in some cases, at least, the evidence seems conclusive that it may exist as an acid ion. It combines with certain bases to form definite salts. As calcium silicate it is sparingly soluble in water, 95 parts per million.

In combination with the alkali bases, sodium and potassium, it forms salts that are indefinitely soluble in water under certain conditions though soluble only with difficulty after being dried. In solution these salts of silica give a strong alkaline reaction like the carbonates. The combination of water with silica, which is known as silicic acid,  $\text{H}_2\text{SiO}_3$ , is in some ways analogous to the combination of carbon dioxide and water. Both are known as weak acids, but in many respects they are altogether unlike.

From what has just been said it may be inferred that in dealing with silica in its relation to the soil solution one finds much that is unknown and baffling. There appears to be some justification for the view that silica, considered as an acid ion, is chiefly involved in those reactions of replacement or exchange that are known to take place between the soil and the basic constituents of the soil solution. Until the facts are more definitely established it is not possible to do more than to conjecture the nature of these reactions and their relationships to the observed changes in the physical condition of the soil. The known facts are at best extremely fragmentary. We may observe that as a result of treating the soil with a solution of a sodium salt certain reactions take place. Some of the sodium disappears from the solution and equivalent quantities of other bases appear in the solution. If the solution is then replaced as by leaching the soil with pure water, the new solution formed from the contact of the water with the soil gives an alkaline reaction and the soil manifests symptoms which we designate as "colloidal."

These phenomena may be explained by saying that when the solution containing sodium was in contact with the soil some of the sodium of the solution entered into combination with the silica of the soil and replaced from combination other bases; also that the silicate compound resulting from this exchange reaction remains insoluble and inert as long as the solution with which it is in contact contains quantities of the strong acid ions—sulphate, chlorine, or nitrate. Finally, if this solution containing one or more of the strong acid ions be replaced or diluted by pure water then the sodium silicate passes from its previously inert or flocculated condition into a dispersed condition. In this latter condition it exhibits the properties of a colloid in that it may form a hydrosol or a hydrogel. In the condition of a hydrogel it absorbs and holds water, thus making the soil impermeable to the movement of water through it, and when its absorbed water is lost by evaporation the gel cements the soil particles together into solid masses.

It should be clearly understood that the above explanation of the phenomena that may be observed from the treatment of a soil with a solution of a sodium salt is proposed merely as a working hypothesis and not as a statement of facts. The facts remain to be established by further investigation.

In view of what is known it seems probable that a better understanding of the causal relationship between the character of the soil solution and the physical condition of the soil awaits further information concerning those silicates of the soil that lie along the border line of solubility.

#### EFFECT OF DILUTING THE SOIL SOLUTION

When the soil of an irrigated field contains so much soluble material that the soil solution is too concentrated for normal plant growth, the only practicable remedy is to apply an excess of irrigation water and carry



a part of the salt away by leaching. This can be done of course only where the soil is permeable to water and where the conditions of under-drainage are such that the soil does not become permanently saturated or water-logged.

From this statement of the case, it might be inferred that the reclamation of so-called "alkali land" is not a very serious undertaking. As a matter of fact, such reclamation has been found to be very difficult if not impossible in some cases. On the other hand, there have been many instances where it has been both easy and successful.

It is naturally a matter of a good deal of importance to be able to determine in advance whether a tract of salty land can be reclaimed at a reasonable cost. The cost of providing drainage outlets and of preparing the land for irrigation may be large. If this expense is incurred and it is then found that the land does not become productive as a result of the work done upon it, the investment is lost.

The essential feature of the reclamation of salty land is the dilution of the soil solution. This is done by applying water of low salt content to the surface of the soil, to soak down through, dissolving out the soluble material or displacing the more concentrated soil solution, and then by providing drainage outlets for the salty water where such outlets are needed. In this process of diluting the soil solution in a salty soil the reaction of the soil to the change in the character of the solution is a matter of the greatest importance. It sometimes happens that this reaction is very slight; that is, that the physical character of the soil, particularly as regards its permeability to water, remains practically unchanged. Unfortunately, this is not always true. There are many examples of attempts to reclaim salty soil by leaching that have been given up because when the concentration of the soil solution was reduced the soil became nearly or quite impermeable to the further movement of water through it.

These differences in the reaction of the soil to the dilution of its solution have led to corresponding differences of opinion as to the possibilities of reclaiming salty land. In some regions one finds that irrigation farmers are confident that such land can be reclaimed if adequate drainage is provided and the land is thoroughly irrigated. In other regions one finds an equally firm conviction that when a tract of land has once become salty it is permanently ruined.

The fundamental reason for these observed differences in the reaction of the soil has not been clearly understood, but more light has been obtained as a result of recent investigations. The reactions that take place in the field may now be duplicated in the laboratory. When this is done it is found that the differences in soil reaction as the soil solution is diluted are associated with differences in the character of the material in the soil solution.

Where investigators working with salty soils have identified only the acids in the solution, they have observed that the reaction toward impermeability has been associated with a high proportion of carbonate. From this it has been inferred that sodium carbonate, or "black alkali," is the chief cause of the impermeability of the soil.

Other investigators who have identified the basic constituents of the soil solution as well as the acids have found that when these bases consist chiefly of calcium and magnesium the soil does not show much



change in its physical character when the solution is diluted. On the other hand, when the dissolved bases are chiefly sodium and potassium the dilution of the soil solution is accompanied by a pronounced reaction of the soil that is manifested in several ways, among which impermeability to the movement of water is one of the most striking.

Before undertaking a detailed discussion of the reactions that take place and the changes that occur in the physical condition of the soil when the soil solution is diluted, it may be proper to cite some examples of changes in the balance of the constituents of the solution that go on during the process of dilution. There are at least three ways in which such dilutions may be made in the laboratory: (1) By digesting similar samples of soil with different quantities of water; (2) by repeated digestions of the same sample, withdrawing a part of the solution after each digestion and replacing it with an equal quantity of distilled water; (3) by leaching a sample of soil with distilled water, analyzing successive fractions of the percolate. While none of these methods exactly simulates conditions that exist in the field, each of them gives results that contribute to an understanding of the reactions that may be brought about by the application of irrigation water to a tract of salty land.

Reference has been made in a preceding chapter to the fact that when samples of the same soil are digested with different quantities of water and a portion of the water subsequently withdrawn for analysis it is found that with some soils the apparent salt content is higher when a large quantity of water is used for digestion than when a smaller quantity is used. This phenomenon might be interpreted as indicating that some of the soluble material of the soil is very slightly soluble and that as more water is used for digestion more and more of this slightly soluble material comes into solution.

This interpretation is not wholly satisfactory, because it can be shown that if the solution obtained by digesting a soil sample with a large volume of water is concentrated by evaporation, precipitation does not occur until the solution has become much more concentrated than the solutions obtained by using a smaller quantity of water for digestion. Furthermore, the known solubility under laboratory conditions of such salts as would be indicated from the constituents identified in the solution is much higher than is obtained from these digestion experiments. The implication is that some part of the soil, presumably the clay, reacts toward some of the soluble constituents in much the same way as water does. The result of this reaction appears to be to retard the passing of these constituents into the soil solution.

A more detailed discussion of these relationships between the soil and its solution may be postponed for the present until more of the pertinent facts have been established. Some of these facts may be brought out by comparing the character of the solutions obtained by various dilutions. It can be shown that the increased dilution of the soil solution with certain types of soil not only increases the apparent salt content of the soil but modifies the character of the solution in so far as this is indicated by the relative proportions of its constituents.

An example of these differences in the quantity and character of the material dissolved from a soil by different quantities of water is shown in Table XVIII. The soil used in this experiment was from an irrigated field in Arizona. The solution of this soil showed only traces of calcium

and magnesium. The soil was of such texture that it was supersaturated with 30 per cent of water. This made it possible to extract with a suction filter enough of the solution for analysis when 100 parts of soil was digested with 40 parts of water. The experiment included digestion of equal quantities of the same sample of soil with quantities of water ranging from 40 parts per 100 up to 2,500 parts per 100. After the soil had been digested with water for 24 hours, with repeated shaking, a portion of the solution was withdrawn from analysis.<sup>10</sup> The total solids were determined by evaporation and the organic matter by igniting the dried residue. The other constituents identified were the carbonate, bicarbonate, chlorin, and sulphate.

TABLE XVIII.—Effect of diluting the soil solution on the balance of its constituents, the total solids being expressed as percentages of the dry weight of the soil and the constituents as percentages of the total solids a

Dilution ratio of water to soil.	Total solids.	Constituents as percentage of total solids.				
		Organic matter.	CO <sub>2</sub> + HCO <sub>3</sub> 2	Cl.	SO <sub>4</sub> .	Total acids.
0.4:1.....	0.76	7.3	9.7	19.3	35.7	64.7
0.5:1.....	.77	7.8	9.7	17.1	34.6	61.4
1.0:1.....	.82	9.7	11.6	14.1	32.7	58.4
2.5:1.....	.87	10.5	12.9	14.0	29.7	56.6
5:1.....	.94	9.9	14.4	14.2	27.5	58.1
10:1.....	.90	12.1	16.3	14.1	25.8	56.2
25:1.....	1.12	15.2	20.1	12.5	20.8	53.4

a Reported by J. F. Breazeale.

The analysis of these solutions showed that the total quantity of dissolved material obtained from a unit quantity of soil increased from 0.76 per cent when 40 per cent of water was used to 1.12 per cent when the 2,500 per cent of water was used. The important constituents of the solution are reported in four groups—organic matter, carbonates, chlorin, and sulphate. When these constituents are reported as percentages of the total solids it is observed that two of them, the organic matter and the carbonates, show increasing percentages with increasing dilutions, while the other two show decreasing percentages. If these constituents were reported as percentages of the original soil it would be seen that while the percentages of chlorin and sulphate are practically the same for all dilutions, the percentage of organic matter increases from 0.055 per cent with 40 per cent of water to 0.170 per cent with 2,500 per cent of water. The increase in the percentage of carbonate and bicarbonate to the dry soil is even more marked, being from 0.074 with 40 per cent to 0.225 at 2,500 per cent.

It is obvious from the results of this experiment why investigators hesitate to make assumptions concerning the concentration or character of the soil solution as it is available to crop plants when their only information is based upon the analysis of extracts made with 5, 10, or even 20 parts of water to 1 part of soil. With such a soil as that used in the

<sup>10</sup> This experiment was conducted and the analysis made by J. F. Breazeale.

experiment just described, 10 per cent of water would be enough to support plant growth. With this quantity of solution in the soil it is not unreasonable to assume that the actual quantity of dissolved material per unit of soil would be even less than is shown with 40 per cent of water, and this solution would contain a smaller proportion of both organic matter and carbonate.

From other observations that have been made on solutions extracted from irrigated soils it seems probable that under ordinary field conditions the soil solution will only rarely show a reaction for normal carbonate, that is to say, will give a pink color with phenolphthalein. On the other hand, it is almost equally uncommon to find a saline soil containing appreciable quantities of sodium salts that does not show such a reaction when it has been leached with pure water.

In the experiment just described the original sample of soil was subdivided and each part was digested once with a certain quantity of water. This method of treatment is one that is used in making analysis of soils, but it does not correspond to anything that ordinarily goes on in the field. The second method of treatment referred to at the beginning of this chapter bears some resemblance to the process of reclaiming a saline soil by repeated copious irrigations.

According to this second method of treatment, a large sample of soil, 3 kgm., was saturated with water, 900 cc., and the mixture was kept in a closed jar for 24 hours with repeated shaking. After 24 hours a part of the solution, 120 cc., was withdrawn by means of a suction filter, and an equal quantity of distilled water was again added to the soil. In this way a portion of the dissolved material was removed with each sample of the solution, and with each withdrawal and replacement the solution became more dilute.

The soil used in the present experiment was of the same type as that used in the previous experiment, though it showed somewhat less soluble material. The extracted solutions again showed only traces of calcium and magnesium, so that these elements were not determined quantitatively. The analytical methods used were the same as those used in the previous experiments.<sup>11</sup> The analytical results of this dilution experiment are shown in Table XIX. The total solids are given as percentages of the original soil and the constituents as percentages of the total solids.

The successive withdrawals of the solution resulted in the gradual reduction of its concentration. This reduction in concentration was accompanied by a change in the proportions of the constituents, as shown in the table, and also by a change in the physical character of the wet soil. It was observed that while the first withdrawals were made without difficulty the later ones were harder to get out, so that after the fourteenth extract the experiment had to be discontinued because the solution could no longer be obtained.

In this experiment, as in the preceding one, it was found that as the soil solution became more dilute the proportion of dissolved organic matter and of carbonate increased while the proportion of the chlorine and the sulphate decreased. The character of the solution at the end of the experiment was very different from that at the beginning. This difference in the character of the solution was also reflected in a change in the physical character of the soil.

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<sup>11</sup> This experiment was conducted by J. F. Breazeale, who also made the analyses.

The conditions that obtain in the field where a saline soil is being reclaimed by leaching may be approximated most closely in the laboratory by using a glass pot, such as is shown in figure 4, or a glass tube partially filled with soil to which distilled water is added at the top. The water percolates downward through the soil, dissolving the soluble material, and is replaced by subsequent additions of water. The soil solution thus replaced escapes below where it may be collected for analysis.

TABLE XIX.—*Effect of diluting the soil solution on the balance of its constituents, the total solids being expressed as percentage of the dry weight of the soil and the constituents as percentages of the total solids*<sup>a</sup>

Extract No.	Total solids.	Constituents as percentage of total solids.				
		Organic matter.	$\frac{\text{CO}_3 + \text{HCO}_3}{2}$	Cl.	SO <sub>4</sub> .	Total acids.
1.....	0.493	6.9	9.4	19.2	29.3	57.9
2.....	.480	7.0	8.8	16.4	32.6	57.8
3.....	.420	8.1	9.1	15.4	31.2	55.7
4.....	.356	7.6	10.3	15.7	31.8	57.8
5.....	.312	8.0	10.0	15.2	31.9	56.1
6.....	.274	8.9	10.7	14.7	31.4	56.8
7.....	.228	10.2	11.5	15.4	29.4	56.3
8.....	.216	10.2	12.0	14.2	29.8	56.0
9.....	.179	10.6	14.0	14.2	28.4	56.6
10.....	.178	11.6	14.5	13.8	26.7	55.0
11.....	.141	17.1	14.9	13.1	27.0	55.0
12.....	.136	14.5	16.0	11.1	24.9	52.0
13.....	.119	16.8	17.0	12.5	22.7	52.2
14.....	.108	16.4	16.0	12.2	19.5	47.7

<sup>a</sup> Reported by J. F. Breazeale.

This method of leaching soils is subject to the same difficulties in the laboratory as are met in the field. With certain soils the dilution of the soil solution is accompanied by a change in the physical character of the soil such that the rate of percolation is reduced to the vanishing point. With soils that show this reaction to a marked degree it is no more practicable to conduct leaching experiments in the laboratory than it is to reclaim them in the field.

It has been observed by a number of investigators that when the soil solution contains a fairly high proportion of calcium and magnesium it is possible to dilute the solution without causing the soil to become impermeable. On the other hand, when the soil solution contains a very low proportion of calcium and magnesium and a high proportion of sodium and potassium the dilution of the soil solution is likely to result in making the soil nearly or quite impermeable to water.

It has been shown that this condition of potential impermeability may be induced in a soil that is naturally permeable by the application of even small quantities of sodium salts. Cummins and Kelley (6) cite a case where the application of sodium nitrate as a fertilizer to the soil of the Citrus Experiment Station at Riverside, Calif., has resulted in making the soil become relatively impermeable to water.

It should be definitely understood that there is a real distinction implied by the term potential impermeability as applied to irrigated soils. The same soil may be actually permeable and potentially impermeable. A soil rarely shows symptoms of impermeability while the soil solution contains a large proportion of dissolved material. The existence in the solution of appreciable quantities of such strong electrolytes as chlorin, sulphate, or nitrate appears to inhibit the soil reaction that makes for impermeability to water. This is true whether the solution is rich or poor in calcium and magnesium. Thus an impermeable soil may be made temporarily permeable by treating it with sodium chlorid or sodium sulphate or by leaching it with a solution of these salts. On the other hand, such treatment would almost certainly make a soil potentially impermeable in that a subsequent dilution of the soil solution, as by leaching the treated soil with distilled water, would induce impermeability.

In view of what has just been said it will be clear that experiments in the dilution of the soil solution by leaching, either in the laboratory or in the field, may be expected to be successful only when the solution contains a substantial proportion of calcium and magnesium. Unless this is the case the experiment is doomed to end in disappointment. Even when this condition is fulfilled it is often found that the rate declines as the leaching goes on. But the decline is not likely to continue unless in the readjustments that take place the preponderance of sodium becomes large.

In the leaching experiments now to be described it will be observed that both the soils used showed large proportions of soluble calcium and magnesium. With one of the soils these proportions continued high to the end of the experiment. With the other it declined as the experiments proceeded.

The soil used for the first of these two leaching experiments was taken from an irrigated field in Arizona. When thoroughly dry it was pulverized and placed in a glass tube of such a diameter that 1,000 gm. of the dry soil occupied about 16 inches of the tube. The bottom of the tube was stoppered with a perforated cork. The column of soil was then leached with distilled water and the percolate was collected in successive fractions of 50 cc. each.

These successive percolates were then analyzed with results as shown in Table XX.<sup>12</sup> The total solids are reported as percentages of the percolate, and the constituents that were identified are reported as percentages of the total solids. As the leaching progressed the solutions obtained became progressively more dilute and the character of the solution was also changed. At first the chief constituents were calcium,<sup>13</sup> chlorin, and nitrate. After the fifth percolate, when 250 cc. of water had passed through the soil, the nitrate practically disappeared. The sulphate content of the first leachings was very low. As the solution became more dilute the actual quantity of sulphate increased somewhat, while the proportion of sulphate to the total salts increased very greatly.<sup>14</sup>

<sup>12</sup> This experiment and the analyses were made by J. F. Breazeale.

<sup>13</sup> The ratio of calcium to magnesium continued throughout the experiment to be approximately as 8 to 1.

<sup>14</sup> Breazeale has observed in another experiment that while calcium sulphate is soluble in distilled water to the extent of approximately 2,000 parts per million, its solubility is reduced in the presence of calcium chlorid. With 26 per cent calcium chlorid he found less than 500 parts per million of dissolved calcium sulphate, while with a saturated solution of calcium chlorid he found calcium sulphate practically insoluble.

TABLE XX.—*Effect of diluting the soil solution by leaching when the solution is rich in calcium and magnesium; 1,000 gm. of soil, 50 cc. fractions of percolate*

Percolate No.	Total solids, <sup>a</sup>	Percentage of constituents to total solids.					
		Ca + Mg.	$\frac{\text{HCO}_3}{2}$	Cl.	SO <sub>4</sub> .	NO <sub>3</sub> .	Total acids.
1.....	37.58	27.1	2.0	35.5	0.2	5.0	42.7
2.....	26.52	35.2	2.3	40.9	.3	5.1	47.6
3.....	21.57	36.2	2.6	41.2	.4	4.9	49.1
4.....	10.95	37.5	4.4	44.7	.9	6.3	56.3
5.....	4.45	28.7	3.0	42.5	4.2	5.9	55.6
6.....	2.82	25.5	1.5	47.2	4.9	2.1	51.8
7.....	2.86	22.4	.8	44.1	4.0	1.4	50.3
8.....	2.04	21.5	1.3	43.0	5.9	Tr.	50.2
9.....	1.34	24.6	2.0	38.0	10.2	0	50.2
10.....	.83	22.8	3.6	30.2	19.8	0	53.6
11.....	.57	19.4	3.2	23.3	30.3	0	56.8
12.....	.41	21.2	6.6	7.5	43.2	0	57.3
13.....	.32	21.6	7.5	5.3	46.8	0	59.6
14.....	.21	24.5	11.5	5.3	45.7	0	62.5
15.....	.18	20.7	9.1	2.2	40.3	0	51.6

<sup>a</sup> In percentage of solution, including organic matter.

The proportion of chlorin remained fairly constant until the tenth percolate when it began to decline rapidly. At this time the concentration of the solution had been reduced from 37 per cent to 1 per cent. When the proportion of chlorin began to decline rapidly the proportion of carbonate and sulphate began to increase correspondingly. This sequence of changes in the character of the solution obtained from the same sample of soil as a result of leaching illustrates what may go on in a field that is being reclaimed by the same process. The leaching results not only in reducing the concentration of the soil solution but also in changing its character. This change in the character of the soil solution appears to be associated with what may be termed the relative motility of the various constituents of the solution. Certain of these, such as the nitrate and the chlorin, move through the soil very readily, while the sulphate and the carbonate are more sluggish.

In the leaching experiment just described the soil solution was at first highly concentrated, and this concentration declined very rapidly with each successive fraction of the percolate. The conditions of that experiment differed from conditions that ordinarily exist in the field in that when the leaching began the soil in the tube was entirely dry. In an irrigated field the moisture content of the surface inch or two may be low, but below that one finds moist soil. The application of water to the top of a column of dry soil brings into solution at once a large part of the soluble material contained in the upper part of the column. As the resulting solution moves downward it continues to take up more soluble material until it reaches the bottom of the column and emerges as percolate. Each successive fraction of the descending column of water would come in contact with soil from which a large part of the soluble material had been removed, and the concentration of each succeeding fraction would be correspondingly reduced.

The conditions of this leaching experiment also differed from conditions in the field in that the soil in the tube had been thoroughly mixed when

placed in the tube and the soluble material was presumably distributed evenly throughout the mass. In the field where water is lost by evaporation from the surface layer of soil there is usually found an accumulation of soluble material at or near the surface. When water is applied this passes into solution and moves downward with the water.

In order to approximate these field conditions somewhat more closely another experiment was set up. The soil used was a permeable sandy loam from the Newlands reclamation project in Nevada. The leaching was done in glass pots, like the one shown in figure 4. Four pots were used, each containing 200 gm. of soil. The soil was first moistened thoroughly and allowed to stand for some time to establish conditions of equilibrium between the solution and the soil. Each pot was then treated with 1 gm. of sodium chlorid which was mixed into the moist surface soil so that it might go into solution. The soil was then leached by adding 10 cc. of distilled water to each pot. The percolate from each leaching was collected below and analyzed, with the results shown in Table XXI.

TABLE XXI.—Effect on the balance of the constituents of diluting the soil solution by leaching, the total salt being expressed as percentage of the solution and the constituents as percentage of reacting values based on the total acids

Leaching No.	Total salts, <sup>a</sup>	Percentage of reacting values.			
		r Ca.	rHCO <sub>3</sub> .	r Cl.	r SO <sub>4</sub>
1.....	0.295	33.4	6.8	2.3	91.0
2.....	1.525	34.6	1.7	80.3	18.0
3.....	1.600	33.2	.8	80.0	19.2
4.....	2.160	30.4	.6	86.7	12.7
5.....	1.840	29.4	.7	87.3	12.0
6.....	1.575	29.4	.8	86.7	12.5
7.....	1.440	29.7	1.0	85.2	13.8
8.....	1.185	24.5	1.2	83.1	15.7
9.....	.905	21.6	1.5	80.0	18.5
10.....	.725	13.3	3.6	75.2	21.2
11.....	.600	15.5	3.1	72.6	24.3
12.....	.480	12.4	5.5	69.0	25.5
13.....	.340	10.1	11.3	67.5	21.2
14.....	.265	8.5	11.8	64.6	23.6
15.....	.230	11.5	20.5	60.2	19.3
16.....	.195	8.9	23.2	60.2	16.6
17.....	.150	8.7	33.4	54.2	12.4
18.....	.127	7.3	34.2	56.2	9.6

<sup>a</sup> Determined by electrical resistance.

While the ratio of water to soil for each fraction of the percolate was the same in this experiment as in the preceding one, the rate of leaching was different in that each successive application of water was made only after percolation from the previous application had ceased. Often the pots stood overnight between leachings, so that diffusion tended to equalize the distribution of the solution constituents and retard the rate of displacement. These conditions resulted in a much slower rate of decline in the concentration of the successive percolates than that shown in the preceding experiment.

The addition of the sodium chlorid to the solution at the beginning of the experiment also caused some significant changes in the solution.



It would be natural to expect that succeeding percolates would show a large increase in the proportion of chlorin and consequent decreases in the proportion of carbonate and sulphate. The actual quantities of carbonate and sulphate in the solution were not at first materially changed. This was not true with the calcium.<sup>15</sup> While the first percolate after the addition of the sodium chlorid showed no pronounced change in the proportion of calcium to the total salts, the actual quantity of calcium in the solution increased from less than 300 parts per million to more than 1,600 parts per million. This reaction by which sodium added to the soil solution tends to replace combined calcium will be discussed later.

As these leachings continued the concentration of the percolates became gradually reduced, the proportion of calcium declined rapidly, the proportion of the chlorin declined also, but less rapidly, while the proportion of carbonate began to increase as the solution became more dilute. Not only did the proportion of carbonate (identified in the solution as bicarbonate) increase as the percolates became more dilute but the actual quantity increased also. The first percolates showed 120 parts per million of  $\text{HCO}_3$ , while the final one showed 450 parts per million.

These various experiments in diluting the soil solution all show that such dilution, by whatever method it is accomplished, does not operate in the same way with the different constituents of the solution. The evidence available appears to justify the inference that there are continuing reactions between the soil and its water, so that the character of the solution is essentially modified as its concentration changes. These reactions between the soil and its water appear to operate not only to influence the character of the solution but also to influence the physical character of the soil in its relation to the movement of water through it. It is well known also that these changes in the physical character of the soil are manifested when the soil is dried. The same physical condition that retards the movement of water through the soil causes the soil particles to become cemented together into a solid mass when the water is lost by drying. Soils that are readily permeable to water usually crumble on drying.

#### EXCHANGE REACTIONS IN THE SOIL SOLUTION

The leaching of a saline soil in the field must be done ordinarily with irrigation water. Such water always contains some dissolved material derived from its contact with the soil. When it is obtained from wells or from most streams the water is itself a soil solution. The character of this solution is a matter of large importance. Its use on the land may result in a complex of conditions wholly different from the one already existing. In this respect again leaching experiments in the field are likely to differ from those conducted in the laboratory.

It has been noted above that certain changes in composition of the soil solution take place when the concentration is changed, as by the addition of pure water. When another solution is added to the one already present in the soil, the reactions that follow involve not only the two solutions but the soil also takes part in them. Certain constituents in the blended solutions may be taken up by the soil and other constituents be released from combination with the soil and pass into solution.

<sup>15</sup> In these solutions the proportion of magnesium was so low as to be negligible.



In the second of the two leaching experiments described on the preceding pages it was shown that the addition of sodium chlorid to the soil solution was followed by a pronounced increase in the quantity of calcium in the solution. This phenomenon, which has been called the exchange of bases, is one that has been reported upon by several investigators.<sup>16</sup>

The exact nature of this phenomenon has not been determined. It has been observed that exchanges take place between the basic constituents of the soil solution and the basic constituents of certain insoluble or very slightly soluble substances in the soil. These exchanges of basic constituents that take place between the solution and the soil are probably more important in their effect on the physical properties of the soil than are those changes in the proportion of the acid constituents that have been discussed above. Until the nature of these reactions is better understood it is not possible to do more than speculate concerning their processes or their exact character. The results may be recognized without difficulty.

When a soil is treated with a solution of a sodium or potassium salt that is more concentrated with respect to these elements than the solution already present, a part of the basic element is withdrawn from the solution and its molecular equivalent of other bases passes into solution from combination with the soil, until a new condition of equilibrium is established. Similar reactions take place when other basic elements are used, such as calcium, magnesium, iron, and aluminum. The consequences of these exchange reactions is to modify the character of the soil as well as of the solution.

These exchange reactions proceed in the direction of establishing a condition of equilibrium between the soil and its solution. It has been found that a limit may be reached beyond which no further reaction takes place. With the soil as with the solution when the limit of saturation is reached reaction ceases. A soil is said to be saturated with respect to a certain basic element when it ceases to show an exchange reaction except when the concentration of that element in the solution is increased.

The results of these exchange reactions on the physical properties of the soil are strikingly different with the different bases. With respect to their effect on the soil the bases that occur most commonly in the soil solution fall into two groups. These groups are sometimes designated as the alkaline and the earthy bases. The alkaline group includes sodium, potassium, and ammonia. The earthy group includes calcium, magnesium, iron, and aluminum. While it may be possible to distinguish between the reaction effects produced in the physical condition of the soil by elements of the same group, these differences if they exist are very slight as compared with the differences produced by representative elements of each. In order to simplify the discussion of the effects produced by the elements of each group on the physical condition of the soil, one element from each may be taken as typical. Thus the alkaline group may be represented by sodium and the earthy group by calcium.

Before proceeding to discuss in detail the effects on the physical properties of the soil of the exchange reactions between sodium and calcium it may be well to establish more definitely the fact that such exchange reactions actually take place. Evidence on this point has been contributed by a number of investigators, but its interpretation has been

<sup>16</sup> See: WAY, J. Thomas (21, 22), and GEDROIZ, K. K. (6). A comprehensive bibliography of this subject is given by Cummins and Kelley (6).

confused by many authors who have held the view that the soil absorbs substances from solution and who have not been clear that such absorption constituted but one part of an exchange reaction. The results of experiments which show quantitatively the extent of such exchange reactions are not numerous, probably because many investigators do not make quantitative determinations of sodium in the course of their experiments. The early experiments of Way (21, p. 332,334) showed that when a soil which contained only traces of water-soluble calcium was leached with solutions of potassium and sodium salts the percolate was practically free from potassium or sodium but instead contained calcium in abundance together with the acid radical contained in the original leaching solution.

More recently Gedroiz (8) has shown that a soil which would give up to 20 consecutive digestions with water (ratio of 5 : 1) only 0.176 per cent of CaO gave from similar treatment with a normal solution of sodium chlorid 1.244 per cent CaO. In a later paper the same author (9) describes an experiment in which a sample of soil was first digested repeatedly with solutions of sodium chlorid until equilibrium was established between the solution and the soil. The soil was then washed, dialyzed, and dried. The treated soil was then analyzed by digestion with 10 per cent hydrochloric acid and its calcium content compared with that of the original untreated sample. The original sample contained 1.36 per cent CaO extractable by 10 per cent hydrochloric acid, while the sample that had been brought into equilibrium with a normal solution of sodium contained only 0.36 per cent CaO extractable in the same way. This result indicates that the absorption by the soil of a certain quantity of sodium from the solution of sodium chlorid had resulted in releasing into solution 1 gm. of CaO or 0.715 gm. calcium from 100 gm. of soil.

The quantity of sodium that was absorbed by the soil during the treatment described was also determined by analyzing samples before and after treatment. These analyses showed that the original soil contained 0.05 per cent Na<sub>2</sub>O, while after treatment with the sodium-chlorid solution it contained 1.42 per cent. This is equivalent to 1.016 gm. of sodium absorbed by 100 gm. of soil.

The absorption of this quantity of sodium was accompanied by the release of other bases than calcium from combination with the soil. Two of these, magnesium and potassium, were identified by the analyses. It was found that the absorption of sodium had caused the release of 0.12 per cent of MgO and of 0.03 per cent of K<sub>2</sub>O. The analytical results of this experiment are summarized in Table XXII.

TABLE XXII.—Percentages of combined (not water soluble) bases in a sample of Russian black earth in its original condition and after being brought into equilibrium with a normal solution of sodium chlorid <sup>a</sup>

Sample.	Constituents soluble in 10 per cent hydrochloric acid, in percentage of dry soil.			
	CaO.	MgO.	K <sub>2</sub> O.	Na <sub>2</sub> O.
Original soil.....	1.36	0.87	0.52	0.05
Treated soil.....	.36	.75	.49	1.42
Replaced.....	1.00	.12	.03	.....
Absorbed.....	.....	.....	.....	1.37

<sup>a</sup> From Gedroiz.

The soil used by Gedroiz in this experiment was rich in combined calcium and poor in sodium. When treated with a solution of sodium chlorid its chemical composition with respect to these bases was profoundly changed. It was observed also that its physical character was changed, but its characteristics with respect to permeability before and after treatment were not reported upon.

Being already rich in calcium it would not be expected that the black earth with which Gedroiz worked would absorb much more calcium and thereby release other bases. The quantity of combined sodium which it contained was so small that there was very little of it to be replaced.

In another experiment reported by the same author a sample of the same black earth was treated with a normal solution of calcium chlorid in the same way as described above for the treatment with sodium chlorid. The sodium was not determined in the second experiment, but the quantities of calcium, magnesium, and potassium extracted from the soil by 10 per cent hydrochloric acid are reported in Table XXIII.

TABLE XXIII.—Percentages of combined (not water soluble) bases in a sample of Russian black earth in its original condition and after being brought into equilibrium with a normal solution of calcium chlorid <sup>a</sup>

Sample.	Constituents soluble in 10 per cent hydrochloric acid in percentage of dry soil.		
	CaO.	MgO.	K <sub>2</sub> O.
Original soil.....	1.36	0.87	0.52
Treated soil.....	1.51	.76	.49
Absorbed.....	.15		
Replaced.....		.11	.03

<sup>a</sup> From Gedroiz.

The results of these experiments show quantitatively the exchange reactions that may take place between the bases combined in the soil and those in solution in contact with the soil.

The fact that such exchanges take place has been observed by several other investigators. It is obvious that such reactions take place in an irrigated field, as will be made apparent by comparing the composition of the irrigation water applied to a field and the drainage water discharged from it.

#### THE COMBINED BASES AND PERMEABILITY

In discussing the reactions of the basic elements that exist in the soil solution it is convenient to recognize two groups—the alkaline, which includes sodium, potassium, and ammonia, and the earthy, which includes calcium, magnesium, iron, and aluminum. The elements of both groups may take part in exchange reactions between the solution and the soil. The effect of such reactions on the side of the soil is to produce one set of physical conditions when the combination is with the alkaline bases and a very different set of conditions when these are replaced by the earthy bases.

These differences in the physical condition of the soil are manifested conspicuously in at least three ways—in permeability to water, in

turbidity of the water extract, and in cementation on drying. When the preponderance of combined bases is with the alkaline group the soil is less permeable to water, the soil extract is more turbid, and the soil tends to cement together on drying. When the preponderance is with the elements of the earthy group these symptoms are manifested in the opposite direction.

It should be kept in mind that the full development of these symptoms in the direction of the alkaline response does not take place while the soil solution contains large quantities of the stronger acid electrolytes, such as chlorin, sulphate, or nitrate. In other words, the presence of these acid electrolytes in the solution tends to prevent the manifestation of the physical effects that follow the replacement of the earthy bases by the alkaline bases in the soil combination. The removal of these dissolved acid ions by leaching the soil or the dilution of the soil solution permits the physical effect of the combination of the alkaline bases with the soil to be shown.

The replacement by an alkaline base of the earthy bases combined with the soil and the effect of this exchange on the permeability of the soil may be observed by following the course of events in a simple leaching experiment. In such an experiment a soil of good permeability should be used. It should be treated with sodium chlorid or sodium sulphate or leached for a time with a solution of one of these. It should then be leached with distilled water to remove the acid electrolyte from the soil solution. An examination of the percolates obtained from the leachings with the salt solution will show that these differ from the solution applied to the soil in that they contain less sodium and that this decrease in sodium is made up by other bases, chiefly calcium. After the leachings with distilled water are begun the percolates become more dilute and the percolation rate becomes much slower and may cease altogether.

The same physical condition of the soil that makes for impermeability to water is also shown by increased turbidity of the water extract of the soil. This may be demonstrated by taking a small quantity of soil used in the leaching experiment just described and shaking it up with water. The use of 10 parts of water to 1 part of soil gives a good example. Another sample of soil taken after the leaching experiment is completed and treated with water in the same way shows the contrast in the turbidity of the extract. These differences in turbidity of the water extract of a sample of salty soil before and after leaching are shown in figure 6. Each of the tubes shown in the figure contained 5 gm. of soil and 50 cc. of water. After the mixture was thoroughly shaken, the tubes stood for two days before the photograph was made. In the tube at the right which contained the unleached soil the solution cleared within a few minutes. In the other tube only the larger particles settled out, the fine material remaining suspended in the solution.

The differences in the cementation properties of a soil in which the earthy bases have been replaced by an alkaline base may be shown by drying a sample from the leaching experiment described above and comparing it with a sample of the original soil that has been saturated but not leached. Such comparison shows that the alkaline treated soil becomes harder on drying than the original permeable soil.

# LEACHING SALINE SOILS IN THE FIELD

In the ordinary practice of irrigation the aim is to apply the water uniformly to the surface of the land. To this end various methods of distribution are resorted to, such as furrows, checks, borders, basins, and contour ditches. In preparing the land for irrigation a good deal of labor is devoted to leveling the surface, so that a uniform distribution of the water may be accomplished. The implication is that if the water is applied uniformly to the surface it will soak into the ground with equal uniformity in each check or along each furrow. As a matter of fact, this does not happen. The soil as it occurs in the field is anything but uniform in texture and in permeability. These differences are often very great at points only a few feet apart. When this is so it is not to be expected that water applied uniformly to the surface of the land will penetrate the soil to the same depth in all parts of the field.

An example of the differences that may be encountered in the penetration of water applied to a well-leveled check is shown in Table IV and illustrated in figure 2. This example is taken from the Newlands Experiment Farm in Nevada. The observations were made at two points in the same check only 15 feet apart. At one of these points the water did not penetrate beyond the first foot, while at the other it reached the third foot and may have passed on into the fourth. Similar instances of differences of penetration may be observed in any irrigated field.

Such differences in the character and permeability of the soil constitute one of the most serious problems in irrigation, both as regards the watering of crops and the prevention or remedy of the accumulation in the soil of injurious quantities of soluble material.

If it were possible to bring about a uniform penetration of irrigation water in the field there would be no such thing as the accumulation of

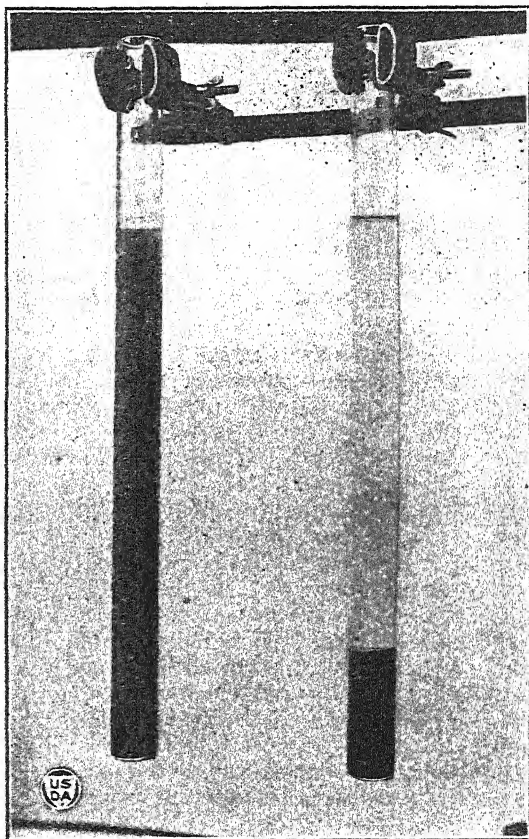


FIG. 6.—Turbidity of solution from the same soil (No. 369) before and after leaching. In each case the tube contains 5 gm. of soil in 50 cc. of water. The tube at the right contains untreated soil which carried a per cent of salt. The tube at the left contains a sample of the same soil after leaching. The removal of the salt by leaching has caused a marked and persistent turbidity.

those soluble substances that cause what is known as the alkali problem. Were it possible to obtain even approximately uniform penetration of water the soluble material would be carried downward with each successive irrigation, and the subsoil accumulations could be removed by artificial drainage where the natural drainage conditions were inadequate.

With conditions as they are, such that the water penetrates readily in some parts of the field or check and very slowly and only to a short distance in other parts, there is a tendency for initial diversities to be exaggerated with the passage of time and with repeated applications of water. The water that is applied to the field carries some dissolved material, often substantial quantities. Some of this water is used by the growing crops, but a large part of it is lost by evaporation from the soil. In either event the dissolved material is deposited in the soil. Where the conditions of permeability are good and an adequate quantity of water is applied there is at least a small surplus to percolate downward beyond the root zone and beyond the range of evaporation. In those spots in the field where the soil is less permeable, the soluble material accumulates in the surface soil or at the lower limit of water penetration, with the result that these spots may finally become unproductive.

The recognition of the existence of such conditions of diversity in the permeability of the soil is essential to an understanding of how irrigated fields become unproductive and why it is often found to be difficult to reclaim such fields even when irrigation water is used in excess of crop requirements and evaporation losses. A field of saline soil may be provided with an adequate drainage system and may be heavily irrigated. There may be a free discharge of water from the drains and yet a large part of the field may remain unproductive. (See Pl. 2, B.)

An investigation of such a situation is likely to show that the leaching effect of the irrigation is confined to those spots where the soil is permeable. In the remainder of the field the water is not moving downward but is held in the soil except to the extent that it is lost by evaporation between irrigations. In fields where the differences in the permeability of the soil are not very pronounced, a protracted period of leaching may be effective in removing the soluble material from all parts of the field. This reclamation may be hastened wherever it is possible to hold the water in place by a system of interior borders that will tend to equalize penetration by holding the water longer on those spots where it soaks in more slowly.

It is well to recognize that there are at least two different sets of conditions involved in differences in permeability. One of these has to do with the texture of the soil, and the other has to do with its physical condition which is largely influenced by the character of its combined bases; that is to say, a soil that is made up largely of clay and fine silt is less permeable than a soil composed largely of sand, providing both types are in the same condition as regards their combined bases. When a check or border includes both types of soil it is well worth while to subdivide it by interior borders which conform to these differences of soil type. Such subdivision makes for economy in the use of water whether for leaching the soil or for irrigating crops.

Where the differences in permeability within a border are due chiefly to the physical condition of the soil resulting from the character of the combined bases it is possible to obtain more uniform penetration of the water by making local applications of salts that will improve the



physical conditions through the exchange reactions described above. For this purpose such salts as calcium sulphate (gypsum) or aluminum sulphate are among the most available. Of these two salts gypsum is the cheaper and more generally accessible. It has the disadvantage of being less soluble than aluminum sulphate and consequently of reacting more slowly.

The beneficial effects that follow the use of gypsum on soils that do not take water readily have long been known, and this salt is extensively used on irrigated land. The assumption that the physical condition of the soil that is associated with slow permeability was due to the existence of sodium carbonate in the soil solution made it natural for earlier investigators to explain the effect of gypsum as a reaction with sodium carbonate. When solutions of sodium carbonate and of calcium sulphate are brought together a reaction takes place by which calcium carbonate is precipitated from the solution. It was assumed that a similar reaction takes place when calcium sulphate is applied to a soil. It is not essential in the present connection to decide whether the calcium applied as gypsum reacts with substances in the soil solution or takes part in exchange reactions with the soil. The obvious fact is that the application of gypsum to a soil that is slowly permeable to water generally results in its improvement.

It has been noted above that gypsum is not very soluble and that its effectiveness is limited on that account. It requires about 500 parts of water to dissolve 1 part of gypsum under favorable conditions. In many situations this low solubility is not a serious disadvantage because the reactions in the soil may go on slowly and be long continued. Where the conditions appear to require more effective action than may be obtained from gypsum it is possible to use calcium in a more soluble form as calcium chloride or calcium nitrate or to use aluminum sulphate. This latter salt which occurs in nature in limited quantities is also manufactured for use in industry and for clearing turbid waters for domestic and industrial use. It has been found that aluminum, like calcium, reacts through the soil solution with the soil to modify its physical condition (9, 15).

In the application of these salts to the soil in the field it would seem advisable to limit their use to those spots where the soil is impermeable rather than to apply them to the whole field. The end sought for is to obtain more uniform conditions of water penetration, and this end may be attained more economically by confining the treatment to the spots that show the need of it. After a field has been irrigated an exploration of it, using a sharp pointed steel rod or a soil augur, makes it possible to locate the spots where the water has not penetrated to the depth desired. These spots may then be given appropriate treatment.

An example of the effect of an application of aluminum sulphate to increase the permeability of the soil is shown in Plate 1. The field in which this experiment was made is at Sacaton, Ariz. A number of borders in this field included spots on which there was no crop growth; though other places in the same borders produced good crops. In the experiment illustrated in the figure two small basins were made side by side in one of the bare spots. To one of these, the one shown on the right, aluminum sulphate was applied at the rate of 5 tons per acre; the other basin was left untreated. The two basins were then irrigated with equal quantities of water about 3 inches in depth. The water

was absorbed in the treated basin within about five hours. The photograph shown in the figure was taken 54 hours after the water was put on. It shows that the water was still standing in the untreated basin while the soil in the treated basin was beginning to dry and crack.

There is another condition of impermeability sometimes encountered in irrigated land that differs from those described above in that it occurs in a zone of limited thickness somewhat below the surface of the soil. This condition, which has been referred to as "hardpan" or "plowsole," is sometimes found in soils that have not been irrigated, and it also develops under certain conditions in irrigated lands (10).

The formation of hardpan appears to be due to the precipitation of substances from the soil solution at the point below the soil surface where evaporation takes place. Such precipitation may be due in part to evaporation and in part to the loss of carbon dioxide from the soil solution. The formation of indurated layers in the soil, sometimes referred to as "caliche," is probably due largely to the escape of carbon dioxide and the consequent precipitation of calcium carbonate. These subsurface impermeable layers often interfere seriously with the penetration of water. When they are not too thick and hard, they are often broken up by deep cultivations; otherwise blasting may be resorted to if conditions justify that expense.

The existence of an underground water table in effect constitutes a barrier to the downward percolation of water through the soil unless the drainage conditions are such that the whole body of underground water is free to move. Furthermore, when the underground water stands close to the surface of the ground, it suffers losses by evaporation, and consequently deposits its dissolved material at the point where it evaporates. This deposition of dissolved material from underground water is one of the most prolific sources of trouble in irrigated land.

The movement of water through saturated soil is usually very slow. Consequently, readjustments of underground water levels that have been disturbed by additions from percolating water are very sluggish. The rate of these readjustments is influenced by the same factors of soil texture and physical condition that have been shown to affect the movement of water through subsaturated soil. In the absence of information to the contrary, we find it natural to assume that underground water seeks its level just as open water does. This is no doubt true, but it does so very slowly. If we make a survey of underground water conditions in an irrigated field, we find that the upper limit of the saturated zone is not level, but that in some places the free water stands much higher than in others.

#### THE ACCUMULATION OF UNDERGROUND WATER

The occurrence of soluble material in irrigated soils in harmful quantities may be taken as a definite indication that the irrigation water does not move downward through the soil. It is obvious that if there were a cumulative downward movement of water the soluble material would be carried away. It does not appear to be essential that this downward movement should be continuous or that any large proportion of the water applied to the soil should pass below the root zone. But it does seem certain that at least occasionally some water should pass on. Unless this is so it is inevitable that in time, either near or remote, the



soluble substances brought in by the irrigation water, together with those set free by soil disintegration, must accumulate to the point of harmfulness. Thus it may be said that for continued successful irrigation farming there must be a cumulative downward movement of water through the soil.

In the preceding chapters attention has been given to certain factors which contribute to a condition of the soil which hinders or prevents the normal downward percolation of water. There remains to be considered another situation which is equally effective in preventing such movement. This is the situation that exists when the subsoil is supersaturated with water. A large part of our irrigated land lies in the valleys of rivers where it is most convenient to divert water for irrigation. These valley lands are very commonly made up of sedimentary deposits from the main stream or its branches or from material eroded from adjacent high land. Only a part of the stream water is carried in the open stream bed. Another part, and often a very substantial part, is carried as underflow below the stream bed and under the adjacent valley lands. This underground water may occur as a continuous body extending to great depth and moving perceptibly though very slowly downstream, or it may be confined to certain underground channels of more permeable material, such as gravel or sand, which lie between islands of clay or fine silt in which no free or moving water is found. The diversion of stream water and its application to the valley lands very often results in adding to the supply of underground water to such an extent that the natural underground drainage ways are inadequate to carry it away. This results in raising the level of the saturated zone and necessitates opening new drainage channels if complete water-logging of the land is to be prevented.

In connection with a leaching experiment made on the Newlands Experiment Farm, Nev., in 1922, some observations were made which bear on this point. The field in which this experiment was conducted was divided into plats of approximately half an acre each. These plats were separated by parallel borders 85 feet apart. A large tile drain had been installed some years earlier along the south side of this series of plats, so that one end of each plat was just above the drain and the other end was less than 300 feet from it. There was a free discharge of water through this drain and the tile was seldom submerged. At the beginning of the leaching experiment the water in the sand box of the drain at the west end of the series of plats stood at 3,955.8 feet above sea level. At the east end of the series it stood at 3,955.5 feet. Though the drainage discharge varied somewhat during the experiment, the height of the water in the sand boxes changed but little. The ground surface in the plats ranged from just below 3,960 to 3,961 feet above sea level. Thus the water in the drain was about 5 feet below the ground.

The ground-water conditions within the plats were observed by means of open wells set in the borders between the plats. There was one line of wells across the north ends of the plats and another line across the south ends. These wells were cased with galvanized iron tubing 3 inches in diameter. Being set in the plat borders they were protected from direct filling when the plats were irrigated. During the progress of the leaching experiment it was possible to observe the height of the ground water as it was shown by these wells and to take samples from time to

time for analysis. The results of one series of these observations are shown diagrammatically in figure 7 for the north line of wells and in figure 8 for the south line.

These plats had been irrigated each week during August and September. Just after the irrigation that was completed on September 30 the water stood in the wells as shown by the upper line in each figure. Six days later, on October 5, the water stood as shown in the middle line in the figures, while nine days later, on October 14, it stood still lower. There are a number of points brought out in these diagrams that deserve mention. Possibly the most striking thing is that the upper limit of the saturated zone was not level or even approximately so. Then, too, the rate of subsidence was very different for the different wells. The variation in this respect was from 0.5 feet to 2.85 feet for the 15-day period. It may also be recalled that the south line of wells was located

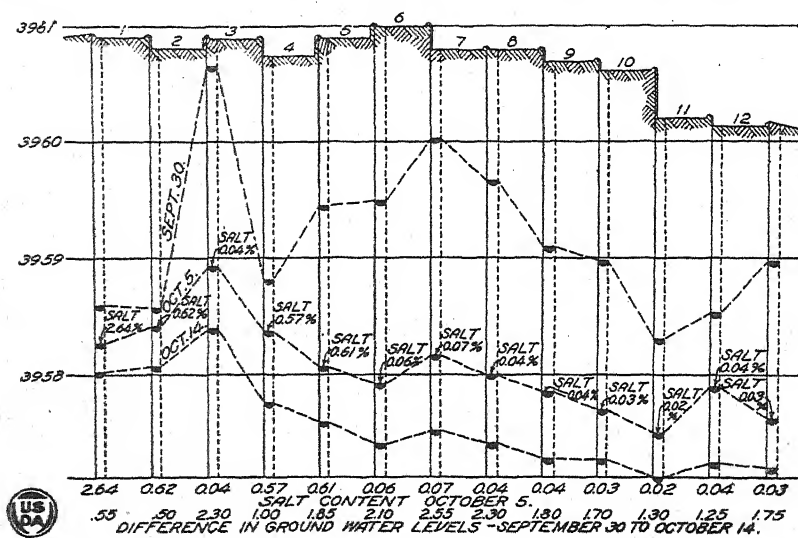


FIG. 7.—Ground-water levels, series Y north, Newlands Experiment Farm; irrigated Sept. 23 to 30, 1922.

only 15 feet from the tile drain in which the water was kept below 3,956. In only two of the wells in this line did the water drop below 3,957. The salt content of the water in these wells as determined on October 5 is shown on the diagram. These figures show that in quality also the water differed from well to well.

In the field in which these observations were made the underground water has been for years not more than 4 feet below the surface. While the soil in many large spots in the field has been so impermeable that irrigation water could not get through it, there have been other spots through which the water has soaked down readily. The lateral movement of the underground water has been very slow, in fact, almost imperceptible. It is probable that the evaporation losses from the moist soil when the water table has been high have resulted in concentrating the soil solution fully as much as it has been diluted by such contributions of irrigation water as have been made to it.

## THE MOVEMENT OF UNDERGROUND WATER

The water that accumulates in the subsoil of an irrigated field is often thought of as similar to bodies of open water, such as lakes or ponds or as a continuous body of uniform quality and that if it is tapped by a drain its quality may be determined by sampling the drainage water just as we would sample a stream that flowed from a lake. If it were desired to know the quality of the water in a lake it would not be thought necessary to obtain samples from many different places in it. There might be slight differences in the quantity or character of the dissolved material as between the surface and the bottom of a lake or between points where springs or streams flowed into the main body of water. In an open body of water such differences are usually slight and they are still less in moving water such as streams. The movement of water results in thorough mixing,

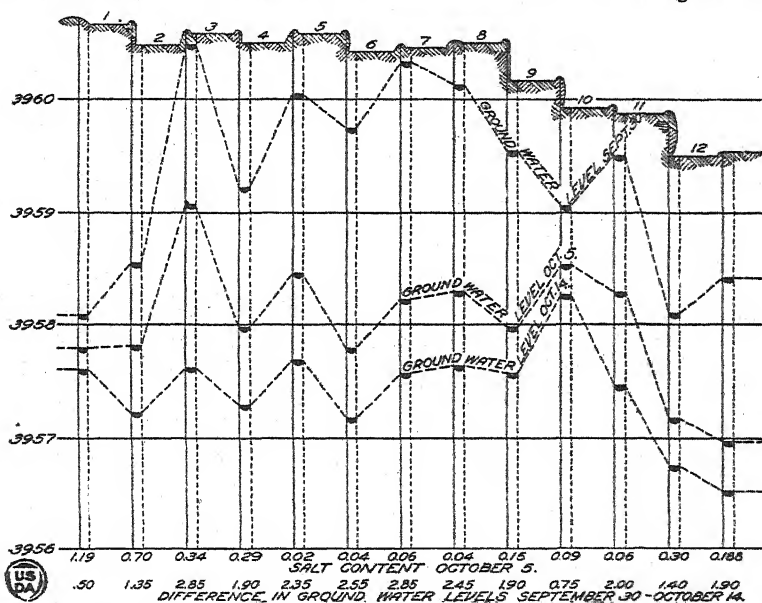


FIG. 8.—Ground-water levels, series Y south, Newlands Experiment Farm; irrigated Sept. 28 to 30, 1922.

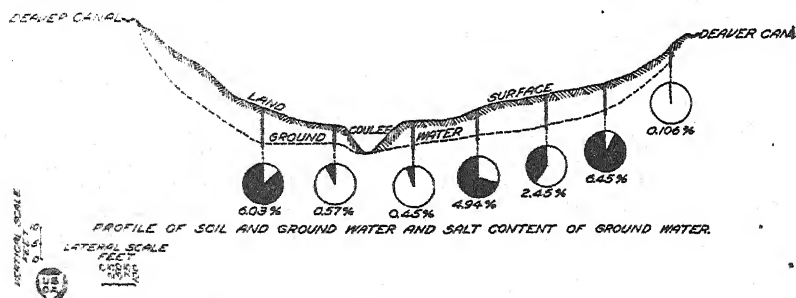
so that a sample taken at one place in a stream is found to be much like a sample taken at another place in the same stream.

When we find that a drain is drawing water from the saturated subsoil of a field we are likely to assume that a sample of this drainage water may be taken as representative of the accumulated subsoil water of that field. As a matter of fact, a more detailed investigation of such a situation would be likely to show that samples of water obtained from the saturated subsoil in different parts of the field contained very different quantities of dissolved material.

The analogy of the drainage discharge from an irrigated field is not with a stream discharging from a lake but rather with a stream discharging from a swamp. In an open lake the water is free to move as in response to the wind, while in a swamp the movement of the water is restricted by islands and other barriers. A stream may flow through a swamp by way of a number of channels and not come in contact or mix with water

held in other parts of the swamp. When evaporation is going on rapidly the water in some parts of the swamp may become much more concentrated than in the channels through which the water is moving freely. In much the same way the water applied to a field by irrigation enters and moves through the underground water in the more permeable channels and passes out in the drainage without necessarily mixing with the water held in the less readily permeable areas of the subsoil.

The actual course and rate of the movement of underground water is not easy to observe. Under some conditions it has been found possible to estimate the rate and direction of the movement of what is called underflow by putting into it at some point, as in a well, a quantity of some substance, such as a dye, which could be identified in samples taken from adjacent wells. Such investigations show that the actual movement of water by underflow is usually very slow. The fact as to whether or not there is a definite movement of underground water with its consequent mixing and something as to the rate and range of such movement may be established by the chemical examination of a series of samples of underground water from any given area. These observations as to the quantity and character of the dissolved substances, together with the fluctuations in the elevation of the underground water resulting from irrigation, give a clue as to the rate and extent of the movement of such



water. Where it is found that the water taken at several points is similar with respect to its dissolved materials and that similar changes in level result from a common cause it may be inferred that there is a free movement. When these observations show pronounced differences it may be assumed there is very little movement or that the movement is confined to restricted zones or channels.

As an illustration of a situation in which there appeared to be very little movement in the underground water, a series of observations made on the Shoshone project, Wyoming, may be used. These observations were made by putting down a line of wells across the valley of a coulee around the head of which the Deaver Canal is located.<sup>17</sup> The canal forms a loop at this point which is crossed by the line of wells as shown in figure 9.

The wells were put down about 250 feet apart. The depth to water ranged from 2 to 10 feet below the land surface. The gradient of the plane of underground water is such as to indicate an absence of equilibrium and consequently a potential movement toward the coulee. In

<sup>17</sup> The writer is indebted to J. R. Iakish, of the United States Reclamation Service, for conducting the field work and collecting the samples of water.

the diagram, which shows a cross section of the ground in line with the wells, the position of the free water in each well is shown in relation to the land surface. The proportion of the dissolved solids, or salt content, of the water of each well is shown in figures below each well and shown also diagrammatically in the circles. It is apparent that the quality of this underground water is very different in the different wells. In the well at the extreme right, which is close to the canal, the salt content is very low. The next three wells show more salty water, while the wells on either side of the coulee are again less salty. The details of the analyses of the samples from these wells are shown in Table XXIV.

TABLE XXIV.—*Quality of underground water on the Shoshone project, Wyoming, from a series of shallow wells 250 feet apart; collected by Iakish and analyzed by Breazeale*

Well No.	Total solids, per cent.	Constituents as percentage of total solids.					Total acids.
		Ca+Mg.	$\frac{\text{HCO}_3}{2}$	Cl.	SO <sub>4</sub> .	NO <sub>3</sub> .	
11.....	6.031	4.9	1.1	2.8	58.0	5.1	67.0
12.....	.572	15.9	3.3	2.0	63.0	.9	69.2
13.....	.454	19.5	9.8	3.4	52.5	Tr.	65.7
14.....	4.948	12.5	1.1	2.9	62.0	.....	66.0
15.....	2.452	24.8	.9	1.4	64.0	.....	66.3
16.....	6.452	12.1	.4	1.4	63.0	.....	64.8
17.....	.106	16.0	22.0	Tr.	49.0	.....	71.0

The results given in Table XXIV tend to confirm the implication of the conditions shown in the figure, which is that the underground water is not moving; at least it is not moving in the direction of its gradient. The samples taken from these wells differ profoundly not only as to quantity of dissolved material but also as to its character. Such differences could not exist if there were much movement of the water with its consequent mixing.

The condition of stagnant underground water such as that just described might be regarded as not unusual when as in this case the land was not irrigated. The frequent application of irrigation water might be expected to result in time in obliterating such differences. The results of another series of observations made in an irrigated field on the Newlands Experiment Farm, Nev., show that even where a field is irrigated frequently and copiously and where there is a drainage system to carry away the surplus underground water the movement of the water is very irregular and its quality is equally variable, at least for a long time. The observations now to be presented were made in connection with the leaching experiment referred to in the preceding pages. The field included 6 acres divided into 12 plats, each 85 feet wide. These plats have been irrigated almost continuously since 1908, but much of the soil has been impermeable and crop growth has been very irregular. A view of the crop growth on one of the plats in this field is shown in Plate 2, A. In 1913 the accumulation of water in the subsoil of this field had reached such a point that drainage became essential and a tile drain was laid along the south side of the field about 5 feet below the surface. Notwithstanding persistent efforts to improve the condition of the soil by careful irrigation and good tillage, accompanied by manuring and the use of gypsum, this

field remains very irregular in productiveness. There has been marked improvement in some spots, but in others the soil is still difficult to work into good tilth and takes water very slowly.

In the early part of August, 1922, two series of wells were put down in this field. One line ran across the north side of the field and the other along the south side. The wells were set in the borders between the plats. The wells of the south line were set about 15 feet in from the tile drain.

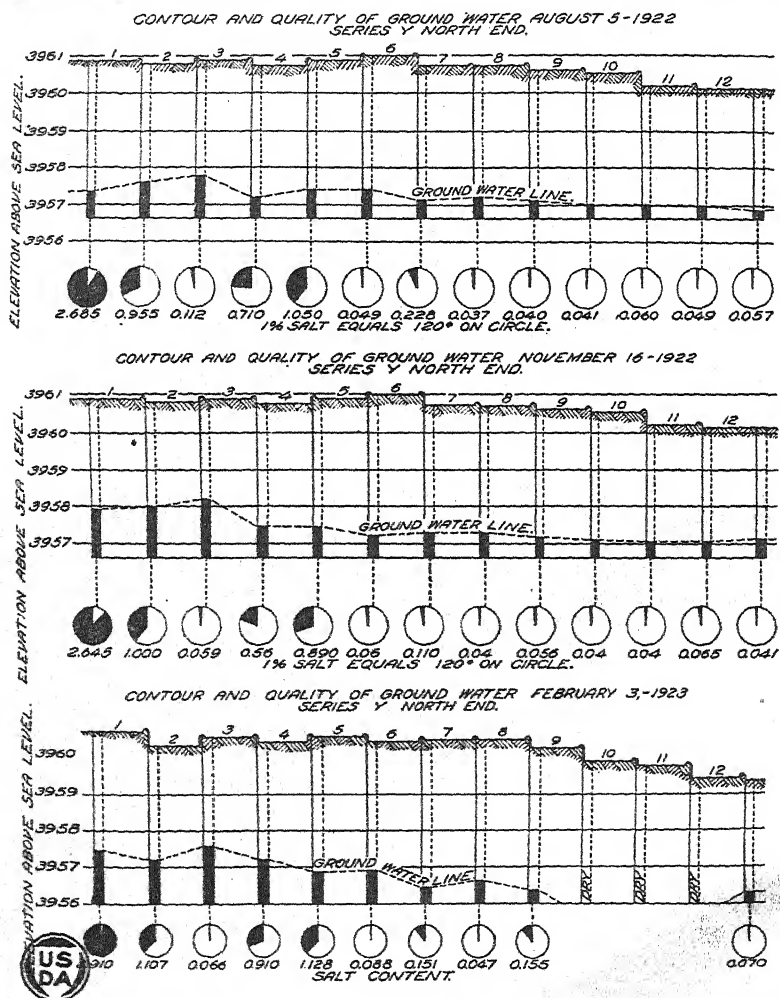


FIG. 10.—Results of experiments on the Newlands Experiment Farm with wells 85 feet apart in the borders between the plats.

These wells permitted observations to be made from time to time as to the height of the ground water, and samples of this water could also be taken. The results of the first set of observations, made on August 5, 1922, are shown in the upper diagram of figure 10 for the north line of wells and in the same position of figure 11 for the south line. These diagrams show the height of the ground water in relation to the ground

surface and also the total salts as determined by electrical conductivity. The salt content is given in the figures and is also shown as segments of the circles. A full black circle would indicate 3 per cent of salt.

In the spring of 1922 the field had been sown to oats and alfalfa. Prior to the beginning of these observations it had been irrigated as the needs of the crop indicated. Irrigation was continued after the observa-

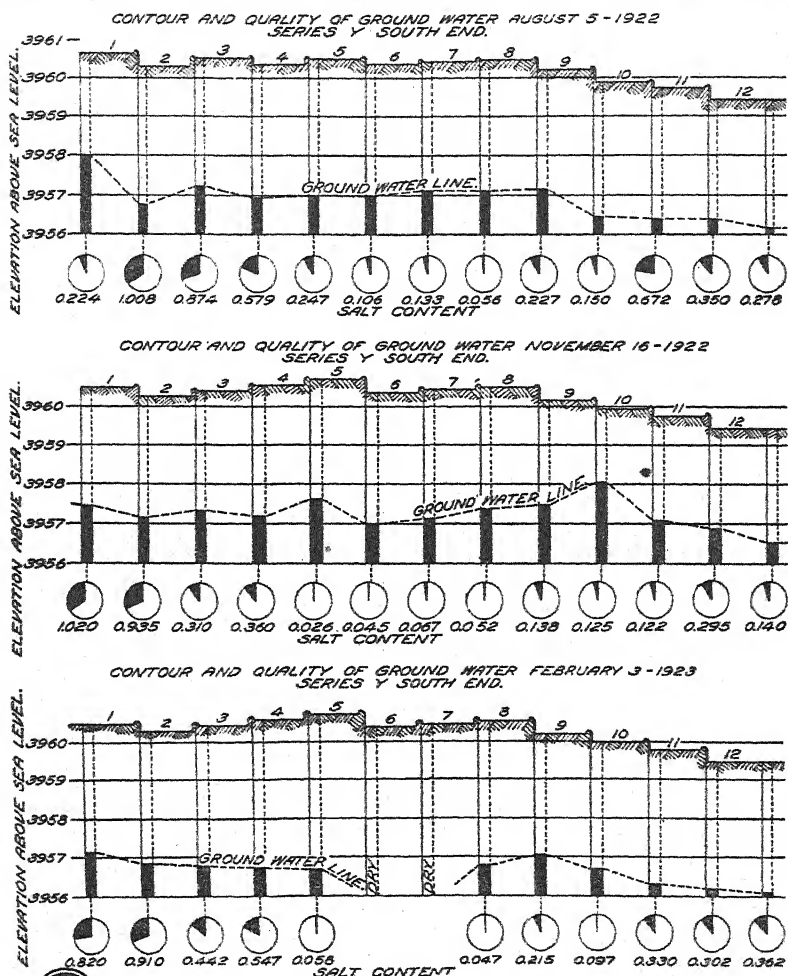


FIG. 11.—Cross section of 12 plats in the south end of the Y series at the Newlands Experiment Farm.

tions were begun, but with the aim of putting on all the water the land would take without injuring the young alfalfa. Nine irrigations were applied between August 5 and October 15. All of the water applied was held on the plats to soak in. In these plats the texture of the surface soil is extremely variable, ranging from coarse sand to a mixture of sand and clay in which the clay predominates. The subsoil conditions are no



less variable, but in putting down the wells it was found that coarse water-bearing sand could be reached, though in some places the clay was several feet thick. Although the wells all penetrated to the water-bearing sand it will be seen from the diagrams that the height of the ground water was not the same in all the wells and that its quality was very different. The range in salt content in the north line of wells was from 2.68 per cent to 0.037. The irrigation water contained about 0.03 per cent of dissolved material.

The diagrams show that the water from some of the wells contained only very little more dissolved material than the irrigation water. The implication is that these wells are located in soil that is readily permeable to water and in consequence has long since given up any excess of soluble material. The immediate effect of irrigation on the height of the ground water in this series of plots is shown in figures 7 and 8. These diagrams

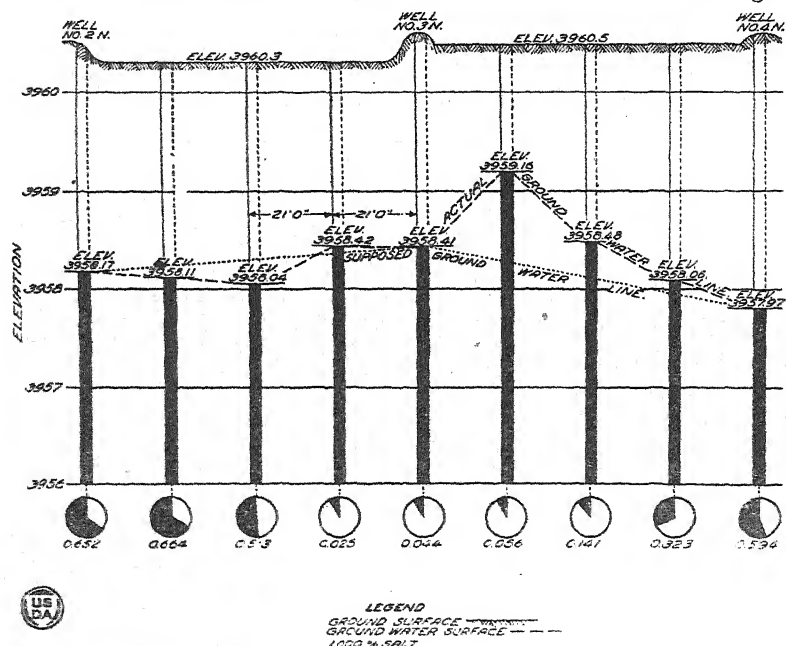


FIG. 12.—Series Y ground waters in wells No. 2 N to 4 N and the intermediate wells on the Newlands project, October 19, 1922.

show that following an irrigation the water stood much higher in some wells than in others and also settled away much faster. Even in the more permeable areas the rate of subsidence was slow, averaging not more than 2 or 3 inches a day for the first week after the irrigation. During the period of frequent irrigations in this leaching experiment when the water was applied as often as once each week the ground water remained about 1 foot higher than it had been before or than it subsided to after the close of the irrigation period.

The condition of height and salt content of the water one month after the final irrigation of the season is shown in the second diagram of figure 10, and figure 11. Although these plots had been copiously irrigated since the first of August and the drain had been discharging water continuously there is seen to be very little difference in the salt content of



each well as between the August and the November observations. There was no irrigation water used on these plats after October 14, and in fact none was used on the farm and very little was used on the whole project after that date. The discharge from the drain continued during the winter with a consequent lowering of the ground water. Observations made on February 3, 1923, gave results as shown in the diagrams at the bottom of each of the figures. In several of the wells the water had fallen below the accumulated sand in their bottoms and samples could not be obtained. These wells are reported as dry. The water in the other wells showed slightly more salt in February than in November. The differences as to salt content between the wells continued with very little change throughout the six months.

The wells in this field were 85 feet apart and all of them penetrated into what appeared to be the same stratum of coarse water-bearing sand, yet the water obtained from adjacent wells showed pronounced differences in salt content. This fact suggested the need of more detailed

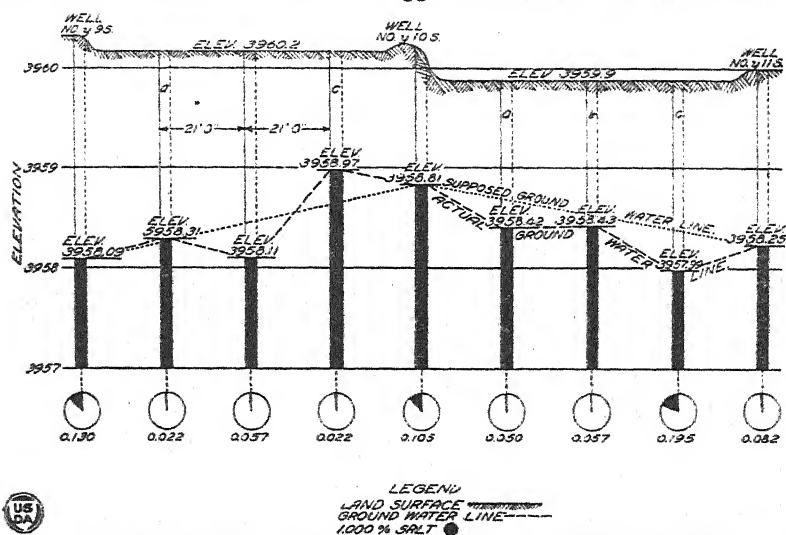


FIG. 13.—Series Y ground waters in wells Y 9 to Y 11 and the intermediate wells on the Newlands project October 19, 1922.

exploration. To this end one section of each line was selected and three additional wells were put down between each adjacent pair of wells already set. This was done at the close of the irrigation season when it was possible to obtain samples of the underground water without danger of direct inflow from irrigation. The new wells were only 21 feet apart. Observations as to depth to water and salt content were then made, with the results as shown in the accompanying figures. Conditions in the north line from well No. 2 to well No. 4 are shown in figure 12, and conditions on the south line from well No. 9 to well No. 11 are shown in figure 13. These results show that even when the wells are only 21 feet apart they show very pronounced differences both as to the height of the underground water and its salt content. The evidence presented from these two sets of field observations is supported by that from many others, all of which point to the conclusion that the movement of underground water is often very slight and nearly always irregular.

This lack of uniformity of the soil in many irrigated fields is very conspicuous. It tends to become more pronounced as the conditions resulting from the accumulation of soluble material become critical. One of the first signs of such trouble is the appearance of spots in the field where crop growth is abnormal. Spots of poor growth or bare spots appear and increase in size as the conditions become worse. These irregularities in the soil are manifested also in the underground water where conditions are such that it accumulates.

In the reclamation of saline soils the same diversity of conditions is found. In some spots improvement is marked and immediate; in others it is much slower. It seems evident that the water used for leaching as well as the water used for ordinary irrigation does not soak down into the soil at a uniform rate in all parts of the field. It is also apparent that the lateral movement of underground water to drainage ways, whether natural or artificial, does not proceed uniformly. There are channels or strata of permeable material in the subsoil often interspersed or cut off by other strata or barriers of less permeable material. In reclaiming land or in irrigating it, one of the most important aims should be to equalize the movement of the water. It is not enough to level the land so that the water may be applied uniformly; it is necessary also to make provision for holding the water for a longer time on those spots in the field where the soil is less permeable.

In planning a system of drainage where the accumulation of subsoil water makes drainage necessary it is no less important to take into account the conditions of permeability in the subsoil. It is not sufficient in laying out a drainage system to consider merely the surface contours of the land. Where a drainage system is designed only to collect and dispose of surface waste waters its location is determined chiefly by surface contour conditions. But when its chief function is to relieve an accumulation of underground water, its location should be determined by underground conditions. Whenever it is possible to do so, such drainage should be designed to intercept water that is moving into a section where trouble exists or is anticipated, as well as to provide outlets from such sections. In making the surveys preliminary to constructing a drainage system to prevent or relieve the accumulation of underground water, it is believed that chemical studies of the water should be helpful in locating the channels of free movement and consequently in placing drains.

#### COMPARISON OF IRRIGATION AND UNDERGROUND WATERS

It has been shown in the preceding pages that when a solution comes in contact with the soil, reactions may take place as a result of which constituents are exchanged. Both the solution and the soil are made different by these reactions. Furthermore, a large part of the irrigation water applied to the land is dissipated by evaporation or absorbed and transpired by crop plants, leaving the dissolved material in the soil. In consequence, the soil solution which accumulates as underground water in irrigated land is likely to be very different in concentration and composition from the irrigation water.

The comparison of the analyses of underground or drainage waters with those of the irrigation water affords a basis of understanding the reactions that are going on in the soil through which the water has passed. Such an understanding is much to be desired because it may make it possible to anticipate and to prevent some of the difficulties that follow

the accumulation of harmful quantities of soluble substances in the soil. Where conditions are such that it is possible to measure or to estimate the quantity of irrigation water used on a certain area of land and to measure also the drainage discharge from the land, analyses of the two waters make it possible to know whether the land is gaining or losing in salt.

Two examples may be cited as illustrations of this point. One is a valley containing about 100,000 acres of irrigated land so situated that the return flow of drainage water may be measured and sampled. Prior to the construction of a drainage system, the land on this valley was badly water-logged. The annual diversion for irrigation purposes aggregated 300,000 acre-feet of water which carried an average of 350 parts per million of dissolved solids. Upon the completion of the drainage system it was found that the aggregate annual drainage discharge was about 100,000 acre-feet of water, carrying an average of 1600 parts per million of dissolved solids. An acre-foot of water weighs 2,716,000 pounds, or 1,358 tons. With 350 parts per million of dissolved solids, an acre-foot of water carries 950 pounds. The incoming irrigation water carried to these valley lands 142,500 tons of dissolved solids, or nearly 1.5 tons per acre. The outgoing drainage, on the other hand, carried away 2.17 tons of salt with each acre-foot, or 217,000 tons for the year, or nearly 75,000 tons more than the irrigation water brought in. This situation indicates a marked decline in the salt content of the irrigated land. The general increase in crop yields in this valley also bears testimony to the improvement of the soil.

In another valley a similar study of conditions shows a different situation. The facts are substantially as follows: The valley includes about 53,000 acres of irrigated land. The annual diversion of water is 200,000 acre-feet, containing an average of 1,000 parts per million of dissolved solids. The annual drainage discharge is about 48,000 acre-feet, carrying an average of 1,410 parts per million of dissolved solids. Thus the irrigation water brings in 272,000 tons of salt annually and the drainage water takes out 92,000 tons, leaving a residue of 180,000 tons of salt in the valley. If this residue were distributed uniformly to the irrigated lands in the valley it would be equivalent to 3.4 tons per acre.

These two examples serve to show how one set of comparisons may be made between the quality of the irrigation and of the underground water. It is not always possible to make such comparisons because the percolating waters from irrigated land are sometimes lost into the country drainage and can not be measured or sampled.

In some situations where it is not practicable to measure the volume of the drainage discharge it is possible to obtain samples of the drainage water and by analysis compare its quality with that of the irrigation water. Such comparison makes it possible to judge of the reactions that are taking place in the soils. If no exchange reactions take place the drainage water should differ from the irrigation water only in being more concentrated as a result of the losses by evaporation or the use by plants. As a matter of fact, it is rather unusual to find a place where the underground water does not differ profoundly from the irrigation water both as to total solids and in percentage composition.

Where the irrigation water carries only a small quantity of total solids and these consist in large part of calcium and bicarbonate it is to be expected that as a result of the aeration and evaporation that takes place when the water goes on to the land these constituents

would be precipitated as calcium carbonate. The remaining solution would then show higher proportions of the other constituents. It is a matter of common observation that underground or drainage waters usually show lower percentages of calcium and of bicarbonate than irrigation waters. An example of these comparisons is shown in Table XXV. This table gives the composition of irrigation and drainage waters from three different irrigated regions. The composition of the irrigation water for each region represents the mean of several analyses. The drainage water from Newlands, Nev., is reported for two locations. One of these, drain Y 13, shows the mean of a series of analyses made on the drainage water from the field on the experiment farm which had been used for the leaching experiment discussed earlier. The percentage of calcium and magnesium in this water is very much lower than that of the irrigation water. The actual quantity of calcium is greater per unit volume of water, which would indicate that the difference in composition of the drainage water must be due in large part to its leaching action. The soil in the field where this drain is located is known to contain large quantities of soluble material the chief basic constituent of which is sodium. The water from the Fernley drain shows a higher proportion of calcium than that from the drain in field Y on the experiment farm. The soil of the Fernley district is known to be much more permeable to water than much of the soil of the experiment farm, and this condition may be associated with a higher proportion of calcium in its soluble constituents.

TABLE XXV.—Comparison of total solids and percentage composition of irrigation and drainage waters from several irrigated sections

Location of sample.	Total solids (parts per million).	Constituents as percentages of total solids.				
		Ca+Mg.	CO <sub>2</sub> + HCO <sub>3</sub> z.	Cl.	SO <sub>4</sub> .	Total acids.
Newlands, Nev.:						
Irrigation.....	158	10.1	34.0	9.5	23.0	66.5
Drain Y 13.....	3,440	1.3	23.6	6.0	28.0	57.6
Drain, Fernley.....	2,720	5.6	6.9	8.4	49.5	64.8
Huntley, Mont.:						
Irrigation.....	278	17.8	24.0	5.6	32.0	61.6
Drain 13.....	5,860	7.8	3.7	1.4	58.4	63.5
Bard, Calif.:						
Irrigation.....	960	17.3	14.5	11.2	37.8	63.5
Field wells.....	1,095	13.8	12.9	15.3	37.3	65.5

The comparison of the irrigation and drainage waters from the Huntley project shows that the drainage water contains lower proportions of the earthy bases, calcium and magnesium, than the irrigation water and also much less carbonate and chlorine. The indications are that in percolating through the soil it is taking up substantial quantities of sodium and sulphate. On both the Newlands and Huntley projects the drainage water is much more concentrated than the irrigation water, carrying 15 to 20 times as much dissolved material.

The waters reported from Bard, Calif., were taken on the Yuma project, which is irrigated from the Colorado River. On this project much of the irrigated land is underlain by a saturated zone that is directly

connected with the water of the river and may be regarded as a part of its underflow. The volume of this underflow is large, but its movement appears to be slow. The field wells from which the samples were obtained that are reported in Table XXV are located on the Bard Experiment Farm. The nine wells are so distributed over the farm of 160 acres as to give samples of the underground water for the whole tract. This underground water probably represents a composite of river underflow and of leachings from the irrigated fields. The figures reported are the means of six sets of analyses from nine wells. These results show a condition where the underground water differs very little from the irrigation water, either in total solids or in the composition of these solids.

The irrigation water that is applied to the land is dissipated in two or three ways. One part of it is absorbed by plants, another part is necessarily lost by evaporation from the soil, and where the quantity applied is more than the sum of these two losses the remainder percolates into the subsoil. The water that is absorbed by the plants is not ordinarily of the same composition as the soil solution; that is to say, the plant roots do not absorb the soil solution with which they are in contact. They absorb the water from this solution and with the water they take up only such dissolved constituents as are needed. This process is known as selective absorption.

It is obvious that crop plants do not absorb the soil solution as it exists in the soil. It is not uncommon to find that the soil solution in irrigated land contains as much as 1 per cent of dissolved mineral matter. It has been found (3) that the ordinary crop plants transpire as much as 500 pounds of water for each pound of dry matter produced. Of this dry matter only 10 per cent or less is mineral matter. From this it may be seen that the plant may transpire as much as 5,000 pounds of water and take into its system only 1 pound of mineral matter. This means that the water that is absorbed and transpired by plants leaves its dissolved mineral matter in the soil almost to the same extent as the water that is lost by evaporation from the soil.

When irrigation is so conducted that the water applied is all dissipated by plant absorption and by evaporation, the salts contained in the irrigation water are then deposited in the soil. Even where the salt content of the irrigation water is low the accumulation goes on and it is merely a matter of time until it reaches critical proportions. It is not uncommon to find irrigation waters in use that contain 1,000 parts per million of dissolved minerals. This is equivalent to 1.35 tons per acre-foot of water. As a basis of comparison it may be said that an acre-foot of soil which contains 15 per cent of moisture holds 300 tons of water. If this water holds in solution 1 per cent of mineral matter there would be 3 tons of such material in each acre-foot. From this it is clear that in the practice of irrigation it is essential that some part of the water applied pass downward through the soil, for it is only in this way that the excessive accumulation of salts may be avoided.

While many crop plants may obtain water from a soil solution which contains more than 1 per cent of dissolved mineral matter, it would appear to be advisable generally to keep the solution below that degree of concentration. In order to do this it follows that in applying irrigation water it should be the aim to use enough so that the proportion lost by percolation is large enough to offset the concentration due to evaporation and transpiration. Thus if the irrigation water contains 1,000 parts

per million of salt enough should be used so that 10 per cent of the quantity applied may percolate below the root zone.

In those irrigated sections where the subsoil is saturated, that is, where there is a water table not far below the surface of the ground, it is readily possible to determine the concentration of the soil solution. The accumulated underground water may be taken as representing that part of the solution that has percolated from the root zone. Where there is a drainage system which relieves the underground water, samples of the drainage discharge are usually more dilute than the average of the soil solution because there is nearly always some waste of irrigation water into the drains, and, furthermore, the downward percolation of water through the soil is not uniform. Where the soil is more permeable there is freer downward movement and less concentration through evaporation and transpiration.

It has been shown earlier in this paper that the detailed exploration of a continuous body of underground water shows great differences in concentration even within short distances. In general it seems safe to assume that where the concentration is high the movement of the water is slow, and, conversely, that low concentration indicates freer movement. Occasionally it is found that the salt content of the underground water is very high even where the surface conditions give no indication of it. Such a situation has been observed on the Huntley Experiment Farm in Montana. In one of the fields which has been continuously very productive a well was put down in the spring of 1922 for the purpose of sampling the underground water and noting its change of level in connection with a series of similar wells in other fields of the farm. In this field there were no surface indications of abnormal underground water conditions. The crops had been thrifty and uniform in growth and the yields had been large. In the early spring, when the well was put in, the water table was found about 10 feet below the surface. It was very salty, about 4 per cent. Repeated samples were taken and the well was pumped out to obtain fair samples. As the season advanced the level of the water rose until it stood within 3 feet of the surface yet the crop of silage corn on the field continued to thrive. Toward the close of the irrigation season the water receded, and during the following winter it stood again about 10 feet below the surface. The course of these changes in level and the salt content of the water as determined each month are shown in Table XXVI. It seems remarkable that good crop growth was possible on land where the water so close to the surface was more concentrated than sea water. The inference is that the underground water at this point represents the accumulated leachings for a long time and possibly from other places and that the rise of the water in this area resulted from pressures due to seepage or percolation in higher lands. The soil solution in the root zone must have remained more dilute than the water obtained from the well. The irrigation water used on this field contains only about 400 parts per million of salt and this with the rain and melted snow doubtless served to keep the solution in the surface soil below the critical limit, which for corn is probably not far from 1.5 per cent. The character of the solution obtained from the well in this field is shown in Table XXVII. Except for its higher salt content, this water resembles that obtained from the wells in adjacent fields and from a drain which serves the district in which the farm is located.

TABLE XXVI.—*Elevation and salt content of underground water in field O at the Huntley Experiment Farm, Montana*<sup>a</sup>

Date.	Elevation, water surface.	Depth to water.	Salt content, <sup>b</sup>
1922.			Per cent.
Apr. 28.....	2,966.4	10.0	4.25
May 31.....	2,966.7	9.7	4.20
July 1.....	2,967.6	8.8	4.17
Aug. 1.....	2,972.8	3.6	4.17
Sept. 3.....	2,971.0	5.4	4.37
Oct. 2.....	2,970.4	6.0	4.20
Nov. 3.....	2,969.3	7.1	4.28
Dec. 4.....	2,968.9	7.5	4.28
1923.			
Jan. 3.....	2,968.1	8.3	3.90
Feb. 5.....	2,967.7	8.7	4.00
Mar. 1.....	2,966.7	9.7	3.76
Apr. 3.....	2,966.8	9.6	3.90
May 1.....	2,966.0	10.4	3.79
June 1.....	2,966.2	10.2	3.46
June 27.....	2,968.0	8.4	3.24
July 25.....	2,970.9	5.5	3.13
Aug. 22.....	2,971.9	4.5	3.99

<sup>a</sup> The ground surface elevation is 2,976.4 feet above sea level.<sup>b</sup> Determined by electrical conductivity.TABLE XXVII.—*Composition of underground water in field O at the Huntley Experiment Farm, Montana, in July, 1923.*

## PARTS PER MILLION

Total solids.	Ca.	Mg.	HCO <sub>3</sub> .	Cl.	SO.	Total acids.
37,080.....	410	980	240	420	25,764	24,424

## REACTING VALUES

37,080.....	20.5	80.5	3.9	11.8	494.0	509.7
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## PERCENTAGE REACTING VALUES

37,080.....	4.0	15.8	0.8	2.3	96.9	100.0
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<sup>a</sup> Collected by Hausen and analyzed by Brezeale.

## THE ALKALINE CARBONATES

In the literature dealing with the problems of alkali soils the soluble carbonates occupy the center of the stage. "Black alkali" has been the *bête noir* of the irrigation farmer as well as of the investigator. Black alkali is usually defined as sodium carbonate while all the other salts in irrigated lands are referred to as white alkali. The carbonates of the alkali bases, sodium and potassium, are readily soluble in water. The carbonates of the earthy bases, calcium and magnesium, are practically insoluble in

water except when the water contains carbon dioxide. When calcium carbonate, for example, passes into solution in water containing carbon dioxide, it is customary to refer to it as calcium bicarbonate and to symbolize it as  $\text{Ca}(\text{HCO}_3)_2$ . This salt, if it exists, is unknown except in solution. When a solution containing it is boiled, the carbon dioxide is driven off and calcium carbonate is precipitated.

The carbonates of the alkali bases may be identified in and produced from nearly all soil solutions and drainage waters of irrigated lands. When the solution also contains carbon dioxide in sufficient quantity to render it neutral to an indicator such as phenolphthalein, these carbonates are said to exist as bicarbonates, for example  $\text{NaHCO}_3$ . It is possible to produce sodium carbonate or sodium bicarbonate by adding carbon dioxide to a solution of sodium hydrate. Both salts are common articles of commerce.

As it occurs in the soil solution in irrigated land, sodium carbonate has been regarded as the cause of serious injury to the physical condition of the soil and as highly injurious to plant growth. Much investigation has been directed toward discovering the circumstances of its formation and means of removing or neutralizing it. The salt has been thought of and referred to as a carbonate. This designation has focused attention on the acid ion  $\text{CO}_3$  and diverted attention from its basic companion sodium. The acid ion  $\text{CO}_3$  is known as a weak ion. This is probably due to the fact that carbonic acid,  $\text{H}_2\text{CO}_3$ , is inert as compared with such acids as hydrochloric and sulphuric. Carbonic acid is formed when carbon dioxide dissolves in water. The oxide of carbon is formed by the union of carbon and oxygen, as in burning charcoal. It may be released also by decomposition of the complex carbohydrates, such as plant tissues. It is assumed that most of the carbon dioxide that occurs in the soil solution is derived from the decomposition of organic matter in the soil.

The compound known as sodium carbonate is said to be a combination of a weak acid with a strong base. In water solution it reacts like the alkaline hydroxide  $\text{NaOH}$  except that when neutralized with a strong acid such as hydrochloric acid half the sodium in solution as sodium carbonate forms a so-called acid salt. The reactions involved may be symbolized as follows:

- (1)  $\text{NaOH} + \text{HCl} = \text{NaCl} + \text{H}_2\text{O}$
- (2)  $\text{Na}_2\text{CO}_3 + \text{HCl} = \text{NaCl} + \text{NaHCO}_3$   
and
- (3)  $\text{NaHCO}_3 + \text{HCl} = \text{NaCl} + \text{H}_2\text{CO}_3$   
or
- (4)  $\text{H}_2\text{CO}_3 = \text{H}_2\text{O} + \text{CO}_2$ .

The reactions that occur in the soil when the soil solution contains sodium carbonate take place in the same way and to much the same extent with sodium hydrate. This fact permits the inference that it is the sodium and not the carbonate that causes the characteristic reactions. This inference appears to gain justification when considered in connection with the phenomena and the reactions involved in the exchange of bases in the soil. It has been repeatedly demonstrated, for instance, that when a soil is treated with a solution of sodium chloride or sodium sulphate some of the sodium goes out of solution and an equivalent quantity of other bases come into solution. If the solution is then removed and replaced with pure water, the soil and the water show the reactions characteristic of the effect of sodium. The soil is deflocculated and the solution becomes alkaline. In view of these facts it may be justifiable



to approach the subject of the alkaline carbonates in their relation to the soil from the basic rather than from the acid side. In such an approach it becomes possible not only to avoid circumlocutions of language but also to see more clearly into the heart of the problem.

In dealing with the subject of alkaline carbonates in the soil solution it should be kept in mind that by the ordinary method of identification it is not the carbonate but the basic hydroxid that is actually determined (see p. 642). The method of titration gives not the quantity of  $\text{CO}_2$  ions in solution but the quantity of basic ions that are in the solution in excess of the quantity of strong acid ions present. Thus, if one takes 50 cc. of  $\text{N}/10$   $\text{NaOH}$  to titrate with  $\text{N}/10$  acid, it will require 50 cc. of the acid to complete the titration whether the solution is kept free from  $\text{CO}_2$  or is saturated with it. In the same way with a soil solution, the quantity of acid that is required to complete the titration with methyl orange is the measure of the quantity of basic ions in the solution in excess of the quantity of ions of the strong acid radicals, such as chlorin, sulphate, or nitrate. The solution may contain much or little  $\text{CO}_2$ ; it will have no effect on the quantity of acid required. The quantity of  $\text{CO}_2$  in the solution does influence the reaction of the solution with respect to the indicators. Thus solution of  $\text{NaOH}$  in water free from  $\text{CO}_2$  shows a pink color with phenolphthalein until the titration is completed. The addition of  $\text{CO}_2$  to the same solution of  $\text{NaOH}$  reduces the quantity of acid required to titrate out the pink color of the phenolphthalein but not the quantity required to complete the titration with methyl orange.

In reporting the analysis of a soil solution it would be quite as correct and possibly less confusing to state the result of the acid titration in terms of excess of bases to strong acids as to give it in terms of  $\text{CO}_2$  and  $\text{HCO}_3$ . This is, in fact, the custom of some analysts. It is not a matter of great importance what designation is used in reporting analytical results, but it is important to understand as clearly as possible what these designations mean. If it is clearly understood that the term carbonate, as used in connection with the soil solution, means the excess of bases over strong acids, there can be no valid argument against its use. There is, however, the possibility of misconception on the part of persons not thoroughly acquainted with the literature of the subject and the analytical methods used. Such persons might infer that the term carbonate implied carbon dioxid in solution.

The soil solution always contains carbon dioxid. This gas in the solution plays a most important rôle not only in the reactions between the solution and the soil but also in the reactions between the soil solution and the plants. It has been remarked above that calcium carbonate is practically insoluble in water that is free from carbon dioxid, but is very appreciably soluble in water containing that gas. There is ample evidence that the solubility of other soil materials also is influenced by carbon dioxid. An experiment recently conducted for the writer by J. F. Breazeale may be cited as an illustration. In this experiment two samples of the same soil were placed in large bottles and treated with water at the ratio of 10 parts of water to 1 part of soil. The mixture was shaken thoroughly and one sample was saturated with carbon dioxid, the other not being treated. Each day the supply of carbon dioxid was renewed in the treated mixture. After five days the solution was filtered off and titrated with acid and tested for calcium. The results of these tests are given in Table XXVIII, expressed in reacting values. That is, the reacting value of the acid required for titration is shown in the first column, and the reacting value

of the calcium is shown in the second column. The third column shows the difference between 1 and 2, which may be taken as the sum of the bases other than calcium existing in the solution in excess of the strong acids.

TABLE XXVIII.—*Effect of CO<sub>2</sub> in solution on the solubility of bases in the soil, expressed in reacting values based on the soil*

Sample.	r Acid required.	r Ca.	Difference.
1. Untreated.....	39.8	15.0	24.8
2. Saturated with CO <sub>2</sub> .....	147.5	85.0	62.5
Increase from CO <sub>2</sub> .....	107.7	70.0	37.7

In the absence of information to the contrary, it may be assumed that the CO<sub>2</sub> in the solution did not decrease the solubility of any of the material in the soil; in other words, that the increase of dissolved bases shown in the table represents a real increase in solubility.

Certain experiments recently reported by Kelley and Thomas (11) afford another illustration of the effect of dissolved CO<sub>2</sub> on the solubility of soil material. In their experiments they digested the soil for one hour with five times its weight of water partially saturated with CO<sub>2</sub>. The results as reported by them are shown in Table XXIX, computed as reacting values. These results show that while only a part of the CO<sub>2</sub> added to the water of digestion is accounted for as carbonate in the solutions, there is a consistent increase in the quantity of total dissolved bases and in the dissolved calcium with increasing quantities of CO<sub>2</sub> used.

TABLE XXIX.—*Effect of CO<sub>2</sub> in solution on the solubility of bases in the soil, expressed as reacting values based on the dry soil<sup>a</sup>*

Soil No.	r CO <sub>2</sub> added to solution.	r Acid required.	Increase.	r Ca.	Increase.
905.....	None.	17.0	.....	0.25	.....
	50	34.5	16.5	6.25	6.00
	100	42.2	25.2	10.00	9.75
	200	60.5	43.5	20.25	20.00

<sup>a</sup> From Kelley and Thomas.

These illustrations of the effect of carbon dioxide in solution on the solubility of the soil material permit the inference that an abundance of carbonate in the soil solution may sometimes be beneficial rather than harmful. If the soil is rich in calcium carbonate, carbon dioxide in the solution brings the calcium into solution. Once in solution this calcium is free to react with and replace any sodium that may be combined with the soil. It has long been known that the application of organic matter to the soil tends to improve its physical condition. It is possible that this beneficial effect may be due in part to the enrichment of the soil solution in carbon dioxide from the decaying organic matter, followed in turn by the solution of calcium and the replacement of combined sodium by the calcium.

Such evidence as is available appears to indicate that the improvement of the physical condition of irrigated soil to the end of making it more readily permeable to water depends upon disposing of the sodium, whether this exists in the soil solution or in replaceable combination with the soil. It seems clear that it is the sodium and not the carbonate of black alkali that causes deflocculation and impermeability in irrigated soils.

#### THE REMOVAL OF SODIUM FROM THE SOIL

The sodium which causes trouble in irrigated soil must be thought of as existing partly as in readily soluble salts and partly as in combination with the soil material. The sodium salts that are readily soluble, such as sodium sulphate and sodium chlorid, may be removed from the soil by leaching, which is in effect merely replacing the solution in the soil by another solution applied at the surface. This is a very simple matter if the soil is readily permeable to the downward movement of water. The sodium that is combined with the soil material and which in such combination is only slightly soluble is the part that is difficult to remove from the soil. It is this combined sodium that causes deflocculation of the soil and consequent impermeability.

The removal of this combined sodium from the soil by leaching is possible only by replacing it with another base. It is possible to replace sodium from its combination with the soil with any other soluble base. There would be no point, however, in attempting to do so with potassium, for example, because such an exchange would not mend matters. Long experience has shown that calcium is the safest, most effective, and most easily available base to use in replacing sodium from the soil. The combination of calcium with the soil induces flocculation and makes for improved physical condition and permeability. Certain other bases, such as magnesium, iron, and aluminum, appear to produce the same effect.

A great variety of methods and of materials have been proposed for correcting the injurious effects resulting from the combination of sodium with the soil. Calcium sulphate, commonly known as gypsum, has long been recommended and extensively used. As a preventive of bad conditions it appears to be altogether satisfactory. As a remedy to be used when bad conditions have developed it is sometimes inadequate because it is only slightly soluble. Calcium chlorid and calcium nitrate are both very soluble, but the first is so deliquescent that it is difficult to handle except in solution and the second is relatively expensive. Iron sulphate is a by-product of certain manufacturing industries and is often to be had at prices that might justify its use in land reclamation. Aluminum sulphate is also a readily soluble salt and aluminum in solution actively displaces sodium from its combination with the soil and improves the physical conditions. With both iron and aluminum there is a possibility that when used in excess colloidal hydroxids may be formed which might cause temporary impermeability.

The assumption that the deflocculated condition of the soil is caused by the existence of alkaline carbonates in the soil solution has led to some attempts to remedy this condition by the use of strong acids. Some experiments have been made with sulphuric acid, which is often very cheap, and elemental sulphur has also been used to some extent. The implication is that elemental sulphur when applied to the soil is oxidized through bacterial action and converted into sulphuric acid. This oxidation process takes place slowly, so that the product seldom reaches

injurious concentrations in the soil. Where the soil is rich in calcium carbonate, the use of either sulphuric acid or elemental sulphur may be expected to result in the solution of calcium with consequent beneficial effects.

It seems certain that when sulphuric acid is applied to the soil either directly or through the use of elemental sulphur, the reactions are not confined to the soil solution. For example, if it were found by analysis that a certain soil contained a certain proportion of dissolved bases in excess of the dissolved acids, that is, carbonates, and a quantity of sulphuric acid equal to that excess were added to the soil, it would be found that only part of the acid had displaced the carbonate from the solution and that the remainder had reacted with the soil to bring an additional quantity of bases into solution. If one were to extract a sample of the soil solution from that soil and add the acid to the solution the carbonates would be completely displaced and the solution made neutral, but when the solution remains in the soil this is not the case. This point deserves special emphasis because it has not been clearly understood. It has been natural to regard the soil as a mass of inert material holding a certain quantity of a solution, very much as a solution is held in a beaker, and that this solution could be titrated in the field in quite the same way as it could be titrated in the laboratory.

The experiments of Kelley and Thomas already referred to afford an excellent opportunity to illustrate this point that the acid reacts not only with the solution but also with the soil. One of these experiments involved the application of elemental sulphur to a sample of soil which was then moistened to optimum condition and kept at room temperature for 15 weeks. The soil was analyzed for all its water-soluble constituents before treatment. After treatment the water soluble  $\text{CO}_3$ ,  $\text{HCO}_3$ ,  $\text{SO}_4$ , and Ca were determined. The analyses before treatment showed that the total of the reacting values of the soluble acids was 169.2 per million of dry soil. The soil also contained calcium as calcium carbonate insoluble in water but soluble in 4 per cent HCl equivalent to a reacting value of 282 per million. Equal portions of the soil were treated with three different quantities of elemental sulphur, as shown in Table XXX, which also gives the quantities of the soluble constituents identified at the conclusion of the experiment.

TABLE XXX.—Effect of elemental sulphur on alkali soil, the sulphur used and the solution constituents being expressed as reacting values based on the dry soil.<sup>a</sup>

Soil No.	Sulphur added.	r Acid required.	Loss.	r Ca.	Gain.	r $\text{SO}_4$ .	Gain.
905.....	None.	17.0	.....	0.25	.....	74.0	.....
	12.5	10.3	6.7	1.70	1.45	86.1	12.1
	25.0	8.5	8.5	3.00	2.75	94.5	20.5
	50.0	7.6	9.4	6.60	6.35	120.7	46.7

<sup>a</sup> From Kelley and Thomas.

The results given in Table XXX show that a large proportion of the sulphur applied to the soil was oxidized into  $\text{SO}_4$ , which enriched the solution correspondingly. In the sample of soil to which 12.5 units of sulphur were added the dissolved sulphates were increased 12.1 units. The solution

extracted from this soil required less acid to neutralize it than the solution from the original soil by 6.7 units. From this it may be inferred that of the 12.1 units of  $\text{SO}_4$  added to the solution, 6.7 units displaced  $\text{CO}_2$  by combining with the bases already in the solution and that the remaining 5.4 units were united with bases newly brought into solution from the soil. Of the 5.4 units of newly dissolved bases calcium constituted 1.45 units.

In the sample to which 50 units of sulphur were added the solution was enriched by 46.7 units of  $\text{SO}_4$ , of which 9.4 units were absorbed in replacing  $\text{CO}_2$  already in the solution, while of the remaining 37.3 units 6.35 may be assigned to the newly dissolved calcium and 31 units to other bases brought into solution from the soil.

It is clear from these results that of the sulphuric acid formed in this soil by the oxidation of sulphur only a part reacted to displace the dissolved carbonate, or, in other words, to neutralize the soil solution.

The conditions of this experiment were such that the carbon dioxide, liberated either from the solution directly or from the soil carbonates as a result of reaction with  $\text{SO}_4$ , could escape from the solution into the air. The solution and doubtless the soil also actually lost  $\text{CO}_2$ .

In another experiment Kelley and Thomas used sulphuric acid on the same soil, but instead of leaving the soil exposed to the air at optimum moisture content for 15 weeks they digested the soil samples for one hour by shaking them with five times their weight of water after adding sulphuric acid. After this digestion the solutions were analyzed for  $\text{CO}_2$ ,  $\text{HCO}_3$ , and calcium. The results of these analyses are shown in Table XXXI computed as reacting values. This table also shows the quantity of acid added to each soil sample and the increase in calcium and other bases brought into solution by the acid, all expressed as reacting values per million of dry soil.

TABLE XXXI.—*Effect of sulphuric acid on alkali soil, the acid used and the solution constituents being expressed as reacting values based on the dry soil<sup>a</sup>*

Soil No.	r Acid added.	r Acid required.	Gain.	r Ca.	Gain.	r $\text{SO}_4$ .
905.....	None.	17.0	.....	0.25	.....	74.0
	12.5	21.0	4.0	5.00	4.75	.....
	25.0	25.0	8.0	12.50	12.25	.....
	33.3	29.0	12.0	15.10	14.85	.....
	50.0	38.5	21.5	28.10	27.85	.....
	100.0	55.0	38.0	65.80	65.55	.....

<sup>a</sup> From Kelley and Thomas.

In this experiment the results show that the addition of acid to the soil solution did not decrease the carbonates, but instead the solution was actually more alkaline after the acid treatment than before. With the first sample reported in the table to which 12.5 units of acid were added, it is to be inferred that the solution was enriched in bases not only by the 12.5 units equivalent to the acid used but also by 4 units more combined as carbonates. This further increase of 4 units of dissolved bases may be explained by assuming that when the acid dissolved the soil carbonates, such as calcium carbonate, the replaced carbonate enriched the soil solution and thus brought still more calcium carbonate into solution.

These two experiments show very definitely that the addition of sulphuric acid to the soil solution in the soil does not give the results that would be expected if the soil were not present. They tend to confirm the view that the soil is not to be regarded as an inert mass in which a solution is suspended by capillarity, as water is held in a sponge, but rather that reactions are going on continually between substances in the solution and substances combined with the soil.

The present hypothesis is that the sodium that causes soil deflocculation and impermeability is the sodium that is combined with the soil and not the sodium that is in the soil solution. If this is correct, the treatment designed to flocculate the soil and improve its permeability should be aimed to replace the combined sodium from the soil by getting it into the solution and then to remove the solution. The difficulty can not be remedied by merely removing the solution.

A soil may be rich in combined or replaceable sodium and consequently impermeable and at the same time contain a large proportion of calcium as calcium carbonate. With such a soil it is to be expected that any treatment that would bring into solution the calcium of the calcium carbonate would favor the reaction by which the combined sodium would be replaced from the soil and thus permit its removal by leaching. If a soil is found to be deficient in combined calcium, it may be necessary to treat it with calcium or some other base, preferably in the form of a soluble salt. In any case, the removal of combined sodium from the soil appears to require the substitution for it of another base, and if the soil is to be flocculated and its condition improved the substitution must be made by an earthy base.

The removal of sodium from the soil solution under field conditions is not a difficult matter if the soil has not absorbed so much sodium as to become impermeable. In other words, it is possible and not very difficult to prevent the accumulation of harmful quantities of sodium in irrigated soils. It is quite another matter to remove combined sodium from the soil in the field when as a result of such combination the soil has become impermeable to water. In dealing with sodium in irrigated soils an old adage may be used even with added emphasis. It would not seem extravagant to say that one ounce of prevention is worth a thousand pounds of cure.

#### SUMMARY

The effective supply of water that may be stored in the soil to last from one irrigation to the next is limited on the one hand by the field-carrying capacity and on the other by the inability of the plants to take water from the soil beyond what is known as the wilting point.

The soil may hold as much as 6 inches of water per foot of depth, but ordinarily its net effective storage capacity is not much above 2 inches per foot of depth.

The subsoil, which is usually more compact than the surface soil, may become saturated when it contains 5 inches or less per foot, and the addition of 1 inch of water to a saturated subsoil may raise the level of the underground water as much as 1 foot.

When irrigation water is applied to the soil it penetrates in part by flowing into cracks and also by way of the small spaces between the particles of soil.

The rate of the penetration of water into a dry soil is influenced not only by the general texture of the soil but even more by the physical reactions of the soil material to water.

The addition of water to a dry soil causes a perceptible change in color, so that it is possible in the laboratory to observe and measure the rate of penetration and to establish the fact that the rate of penetration is very different in different soils.

In a moist or saturated soil the movement of water, sometimes designated as percolation, may also be measured with a fair degree of accuracy.

The physical condition of the soil, which influences so profoundly the movement of water through it, is largely the result of chemical reactions that take place between the soil particles and the salts dissolved in the soil solution.

The character of the soil solution is a matter of fundamental importance in irrigated farming, not only because of its relation to the plant, but also because of its relation to the physical condition of the soil.

It is difficult to estimate the normal concentration or composition of the soil solution from samples obtained by digesting the soil with an excess of water.

The composition of the soil solution is determined by measuring its more important constituents, which are identified as elements or ions and not as combined salts.

The important constituents ordinarily identified in the soil solution of irrigated lands include the earthy bases, calcium and magnesium, also the acid ions, carbonate, bicarbonate, chlorin, sulphate, and nitrate. The alkaline bases, sodium and potassium, are not usually identified quantitatively, but are estimated by difference.

The results of analyses of the soil solution may be stated in several ways, though for the sake of better understanding, it seems advisable to report only the constituents that have been identified.

There are great differences in the solubility of the various constituents of the soil, and these solubilities are influenced to some extent by the composition of the soil solution.

Changes in the concentration of the soil solution induce reactions between the solution and the soil, and consequently cause changes in the physical condition of the soil.

Changes in concentration also result in changing the balance of the solution constituents in relation to each other.

The soil solution is continually changing in concentration and in composition, and the soil takes part in these changes through reactions between the basic constituents of the solution and the bases combined with the soil.

The physical condition of the soil and particularly its permeability to water is largely influenced by the character of the bases that are combined with the soil. When the alkaline bases, sodium and potassium, predominate the soil is deflocculated and impermeable. When the earthy bases, calcium and magnesium, are in excess the soil is flocculated and permeable.

When saline soils are leached to reduce the concentration of the soil solution it is often found that they become impermeable to water. This condition is due to the effect of the alkaline bases combined with the soil, which causes deflocculation to take place when the salts of the strong acids, sulphate and chlorin, are removed from the solution.

Where the subsoil of an irrigated field is saturated with water or contains strata or barriers that are slowly permeable to water, effective leaching of the soil is seriously hindered because of the accumulation of underground water.

The lateral movement of underground water, like the downward movement of water through the soil, is subject to great variation, depending upon local conditions, and the quality and concentration of the solution are also highly variable even within the same field.

The comparison of the quality and concentration of underground waters from different parts of the field with those of the irrigation water permits a better understanding of the drainage requirements of the soil than is possible from observing only the depth to the underground water.

The injurious effects that have been ascribed to sodium carbonate or "black alkali" in irrigated soils appear to be due to the sodium rather than to the carbonate, and sodium in solution even when associated with the stronger acids combines with the soil and ultimately causes deflocculation and impermeability.

The readjustment of the relative proportions of sodium and other bases in an impermeable soil, to the end of improving the physical condition, depends upon replacing the sodium with another base, such as calcium or aluminum, which, when combined with the soil brings about a flocculated and permeable condition.

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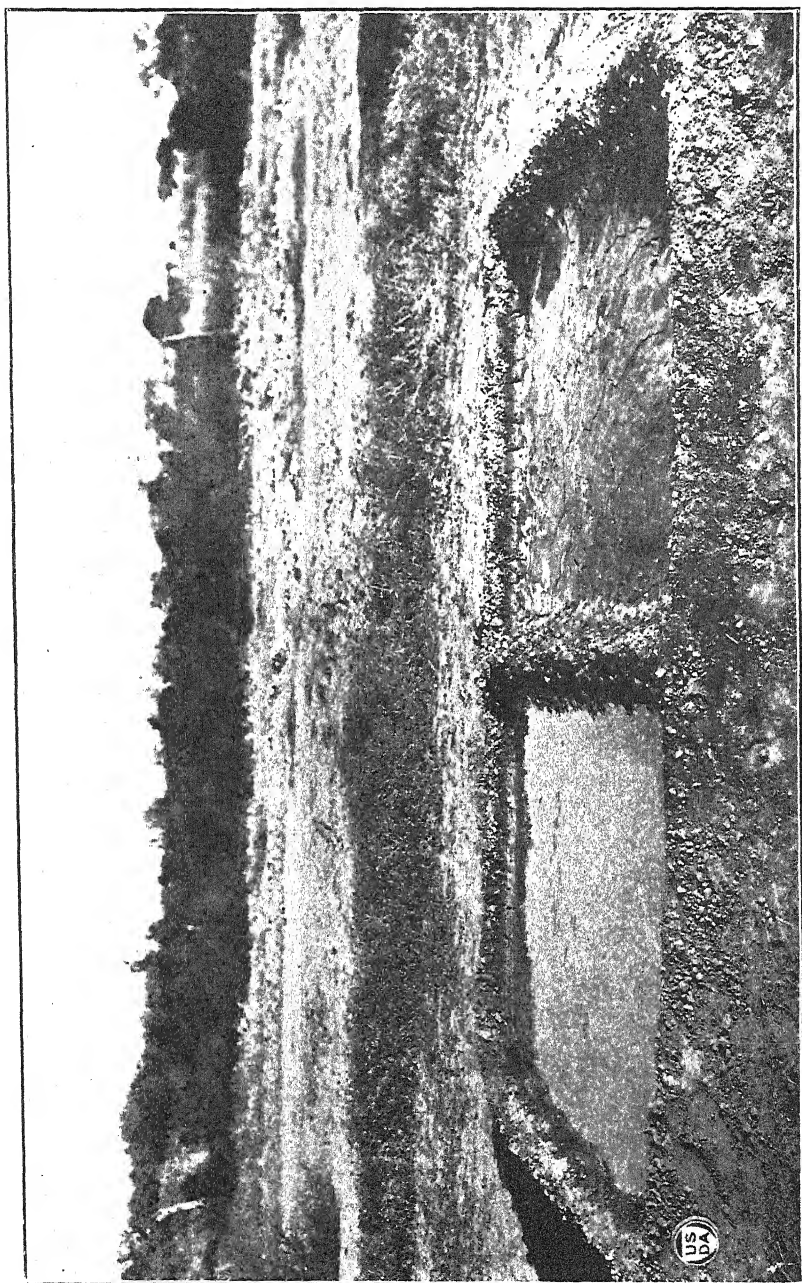
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PLATE I

The effect of aluminum sulphate on the penetration of irrigation water. The plat at the right was treated with aluminum sulphate at the rate of 5 tons per acre, but the plat at the left was not treated. Both plats were given the same quantity of water. Photographed 54 hours after the water was applied; Sacaton, Ariz., November 18, 1921.



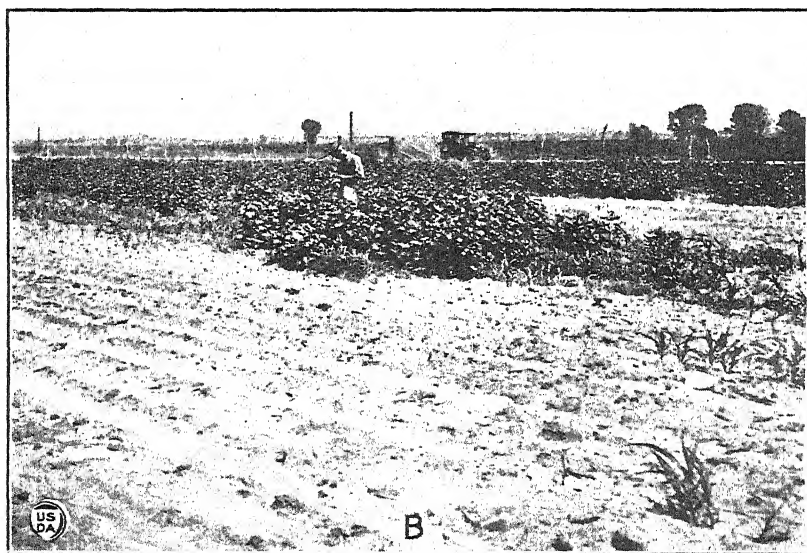
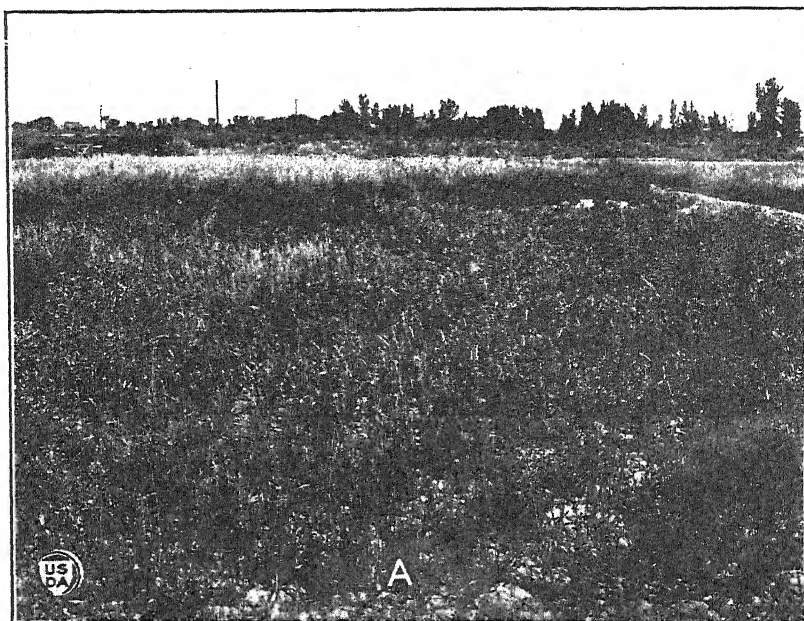


PLATE 2

A.—Irregularity of crop growth on a plat in field Y, at the Newlands Experiment Farm, 1922.

B.—Irregularity of crop grown in cotton field on the Yuma reclamation project, Arizona, 1919.



# AN APPLE STEM-TUMOR NOT CROWNGALL<sup>1</sup>

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Department of Agriculture

There are many kinds of apple-tree outgrowths or stem deformities; some are due to winter injury, some to weak variety conditions, some trees have short internodes, others, swellings of internodes or nodes. The type referred to in this paper as stem-tumor is the outgrowth with masses of rootlike projections or, as it occurs on some varieties of apple trees, an outgrowth with a smooth or nearly smooth surface. The outgrowths vary in size from small warts (Pl. 2, A) to tumors 2 to 6 inches in diameter, though they project scarcely more than one-half inch beyond the surface of the stem (Pl. 1, C). On some trees the bark remains comparatively smooth and the tumors do not reach a breadth of more than two or three inches (Pl. 3, B); but usually the bark breaks and masses of aborted roots appear, giving a roughened budlike surface to the outgrowth. The tumors of the bud type increase in size readily and in time the bark may become black and rough and broken if the tree is a very susceptible one. This may extend along an entire limb (Pl. 2, C). The budlike type is the more common.

These tumors are similar in macroscopic details to tumors produced by artificial inoculation with pure cultures of *Bacterium tumefaciens*. Furthermore, crowngall and hairy-root are widely prevalent on apple trees. Accordingly, although some entomologists<sup>2</sup> have referred to infestation by woolly apple aphid as capable of resulting in galls or warty swellings on twigs, limbs or trunk of apple trees, nursery inspectors, nurserymen, orchardists, plant pathologists and entomologists have in general referred to this type of outgrowth as aerial crowngall. However, this so-called aerial crowngall, of which Plate 1, A, is a good representation, is seldom, if ever, caused by the crowngall organism under natural conditions.

Since 1906 the writer has been concerned with crowngall on various hosts and during this period the attempts to isolate *Bacterium tumefaciens* from apple stem-tumors always have been unsuccessful, but as the tumors resembled the hairy-root type of crowngall as it occurs on apple roots it was believed that the disease was a form of crowngall. In 1923 the writer began a more critical study of the disease.

Some of the material used was received from several different States, and some of it was collected near Washington by the writer, so that there was ample material in good live condition for the platings. Platings were made from tumors just starting to form, and in all stages of growth and development, the largest being 6 inches in diameter.

<sup>1</sup> Received for publication Dec. 30, 1923.

<sup>2</sup> BAKER, A. C. THE WOOLLY APPLE APHIS. U. S. Dept. Agr. Rpt. 101, p. 34. 1915.

CONNOLD, Edward T. BRITISH VEGETABLE GALLS. p. 100. London. 1901.

ORMEROD, Eleanor A. HANDBOOK OF INSECTS INJURIOUS TO ORCHARD AND BUSH FRUITS WITH MEANS OF PREVENTION AND REMEDY. p. 1-6. London. 1898.

STEDMAN, J. M. THE WOOLLY-APHIS OF THE APPLE. In Mo. Agr. Exp. Sta. Bul. 35, p. 40. 1896.

THEOBALD, Fred. V. THE INSECT AND OTHER ALLIED PESTS OF ORCHARD, BUSH, AND HOTHOUSE FRUITS AND THEIR PREVENTION AND TREATMENT. p. 141-153. London. 1909.

During the year 1923, 378 plates were poured. These plates were made from 23 different lots of material and included 38 sets. Previous to 1923, during the years 1909 to 1922, inclusive, there were 120 plates poured from apple stem-tumors, 12 sets from 9 different lots of material. No single colony of *Bacterium tumefaciens* appeared. There were some fairly constant bacterial colonies closely resembling crown gall colonies which appeared on the plates and these were tried out in 1923 on Early Harvest apple trees, a variety known to be susceptible to this disease. The trees were inoculated in May in 138 young growing stems, using 8 different colonies; controls were held at the same time and the inoculations were made in two orchards 18 miles apart. The following greenhouse plants, known to be susceptible to *Bact. tumefaciens*, were also inoculated with these 8 colonies: 30 Paris daisies, 31 young tomato plants, 16 Ricinus, 12 Bryophyllums, 5 tobacco plants and 6 geraniums. The results with these plants were all negative.

In the case of the apple trees under observation, swellings occurred in the axils of leaves of branches where there were neither inoculations nor control pricks. These swellings were small; even as late as October they were only 3 mm. to 1 cm. in diameter. Five swellings which occurred on the main stem of a young tree were much larger; these varied from 1 to 2½ cm. in diameter. The large outgrowths had the typical budlike projections of apple stem-tumor. These places were not inoculated nor were any places on the main stem of the tree inoculated. The only stimulating agent present in October which might be held responsible was the woolly apple aphid (*Erisoma lanigera* Hausmann) a colony of which was on each of these swellings. The tumors had been noted in July when they were smaller, and the aphid was also on them at that time. These tumors were so fresh and soft that the writer, thinking it might be a natural infection of crown gall, cut off portions of two of them expecting to isolate the crown gall organism very readily from these pieces. It could not be done.

Woolly aphids were in the axils of the leaves where axil swellings occurred (Pl. 3, A). The inoculated places were carefully examined and in all of the 138 inoculated stems there were only three outgrowths. These three places were in the axils of leaves and were covered with woolly aphids. Some of the small axil swellings on these Early Harvest apple trees were cut off, and also pieces of the large stem-tumors, a second time. Platings were made from both lots but neither the crown gall nor any other constant organism appeared on the plates.

In an orchard where stem-tumors were quite prevalent 44 trees were examined at the roots and woolly aphid root galls found on 34 of them; 32 of these rootgalled trees had stem-tumors, and only one tree was found with stem-tumors which did not also have the aphid rootgalls. On the roots of one of the trees most severely affected with stem-tumor the woolly aphid was present in great numbers and had produced root-tumors (Pl. 3, C). The same insects were present on the branches also and were abundant on the stem-tumors (Pl. 3, D). No crown gall was found on any of the trees.

Not all varieties of apple trees appear to be subject to these stem-tumors. The character of the soil and cultivation may have much to do with this, but some varieties, as the Early Harvest, seem susceptible under any conditions. Very susceptible varieties which have come under the observation of the writer are the following: Early Harvest, Wagoner,



Martin, Buckskin, Grimes, Ben Davis, Barnes' Best, Ensee. There were 10 varieties in an orchard examined which were less susceptible than the above named in varying degrees. All had woolly aphid rootgalls. The stem-tumors varied in size and number (Pl. 2, B and C) and on some trees there were indications that the tumors had kept on developing for some years; then some change in conditions took place and the newer branches were clean, since few or no tumors had formed during the last years. Four apple varieties in this orchard, Titavka, Loy, Bethlehemite and Van Hoy, had no stem- or root-tumors and did not seem susceptible. The first three varieties had no woolly aphids on them anywhere. Although Van Hoy had some of the woolly aphids on the young twigs there were no swellings.

In a young orchard consisting of 58 apple trees, two of which were the Early Harvest variety, these two were the only ones with the woolly apple aphid on the stems and the only ones with stem-tumors.

Other varieties, such as the Chenango, Spitzenberg and King, affected with stem-tumors, were received from various parts of the United States.

A bad feature of the numerous stem-tumors on apple trees is the opportunity it provides for secondary organisms to get into the stem and ultimately cause more damage than the stem-tumor itself. The tumors are composed partly of soft cortex tissue which wood-invading insects can feed on and get through into the deeper portions of the stem, continuing their work of invasion throughout the year and forming great holes. The movement of sap is, of course, interfered with, which in turn affects the whole life processes of the tree. The breaks in the surface of the bark where the tumors push through likewise offer an entrance for the pear-blight organism, *Bacillus amylovorus*, which also causes greater damage than the stem-tumor (Pl. 2, D).

From the report of the results of Dr. J. J. Taubenhaus' work<sup>3</sup> with apple stem-tumor, it seems that *Bacterium tumefaciens* may occasionally cause stem-tumor on apple tree perhaps similar in appearance but of different origin from that described in this paper.

It is theoretically possible for *Bacterium tumefaciens* to produce tumors naturally on apple stems as well as on the crown and roots, for galls have been produced artificially by inoculating apple stems with *Bacterium tumefaciens* (Pl. 1, B). So far as the writer's observation goes, this organism confines its work in nature to the production of galls and hairy-roots on both crown and roots of apple trees. The cases of apple stem-tumor from various parts of the United States examined by the writer did not contain *Bact. tumefaciens* in a single instance.

#### CONCLUSIONS

Outgrowths on stems of apple trees herein described, and heretofore generally believed to be due to infection by *Bacterium tumefaciens*, the organism producing typical crown gall, are not secondary outgrowths from tumor strands nor are they primary infections of the crown gall organism. Although such conditions have not been observed by the writer, it is possible that in some regions there may be aerial tumors for which the crown gall organism is responsible.

<sup>3</sup> ADAMS, J. F. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1922. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul., Sup. 28, p. 368. 1923. (Mimeographed.)

The root projections on the stem-tumors carrying darkened and usually infected tissue deep into the tumors, and the hard woody nature of the outgrowths themselves, make isolations difficult. When *Bacterium tumefaciens* did not appear on the isolation plates, it was thought due to the dry or old condition of the material used, and it was not until 1923, when young, tender stem-tumors were studied along with older tumors, that the conclusions given in this paper were reached.

Whether or not the woolly apple aphid is the primary cause of the stem-tumors on mature apple stems herein described has not been within the scope of this investigation. There is a possibility, of course, that an organism may be transmitted by the aphids into the stems of apple trees—an organism which undergoes a change through insect transmission and which on that account does not retain its ability to infect after it has been isolated. This might explain the appearance on agar plates of colonies isolated from apple stem-tumor which at first so strikingly resemble the crown gall colonies, but lose this resemblance when cultured artificially and which also fail to infect when inoculated into apple trees and other plants susceptible to *Bacterium tumefaciens*. Had crown gall-infected apple trees been in the neighborhood of the apple trees affected by stem-tumor, the writer would be inclined to give attention to this possibility. But she found none. It seems, therefore, that *Bact. tumefaciens* is eliminated from the consideration.

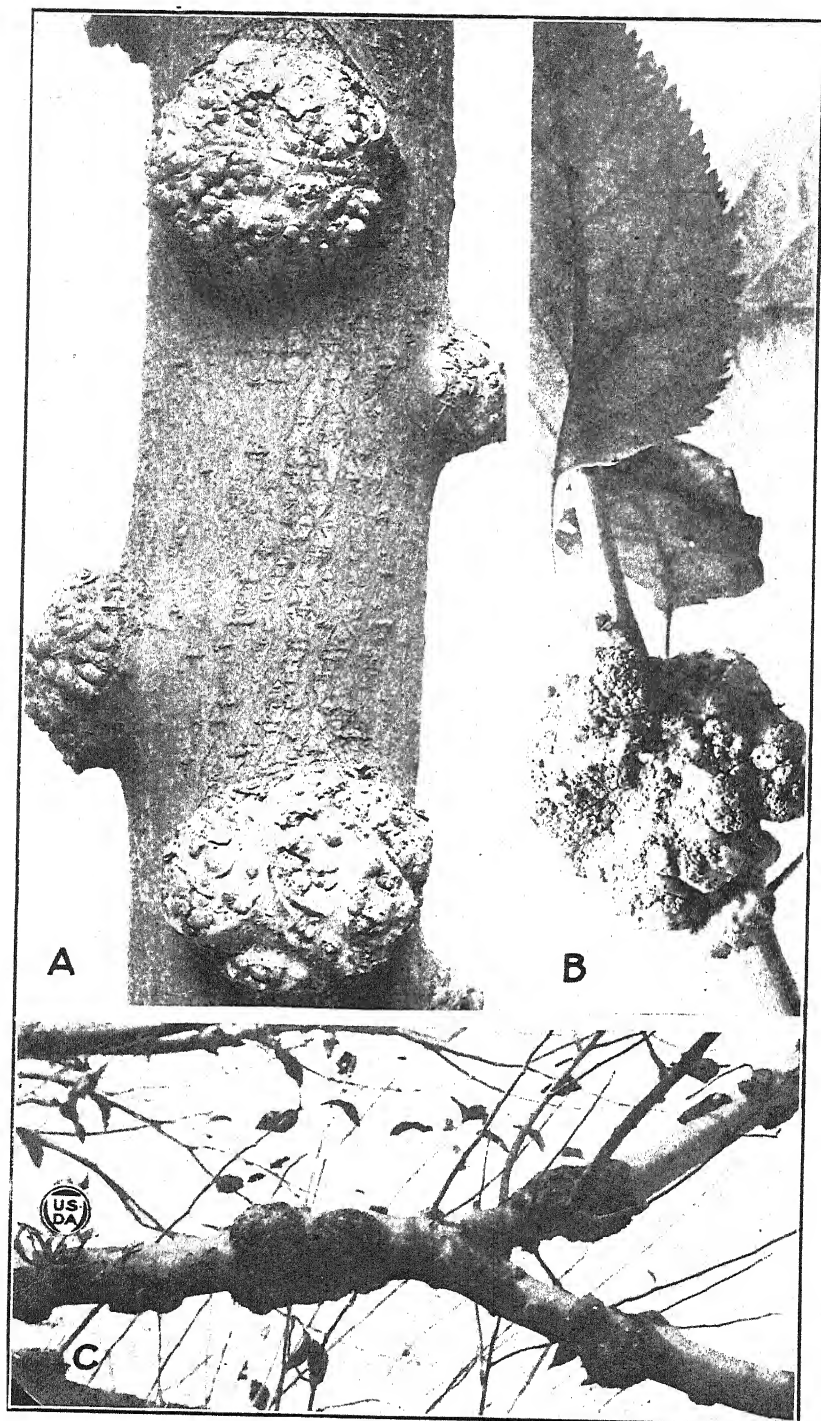


PLATE I

A.—Stem-tumor of apple tree from West Virginia. Natural size. Photographed April 22, 1923. No crown gall organism could be isolated from these tumors.

B.—Crown gall on apple stem produced by inoculating with *Bact. tumefaciens* (peach strain). Inoculated March 11, 1908. Photographed August 10, 1908; three-fourths natural size.

C.—Old and young stem-tumors on Wagoner apple tree, Virginia; one-fifth natural size.



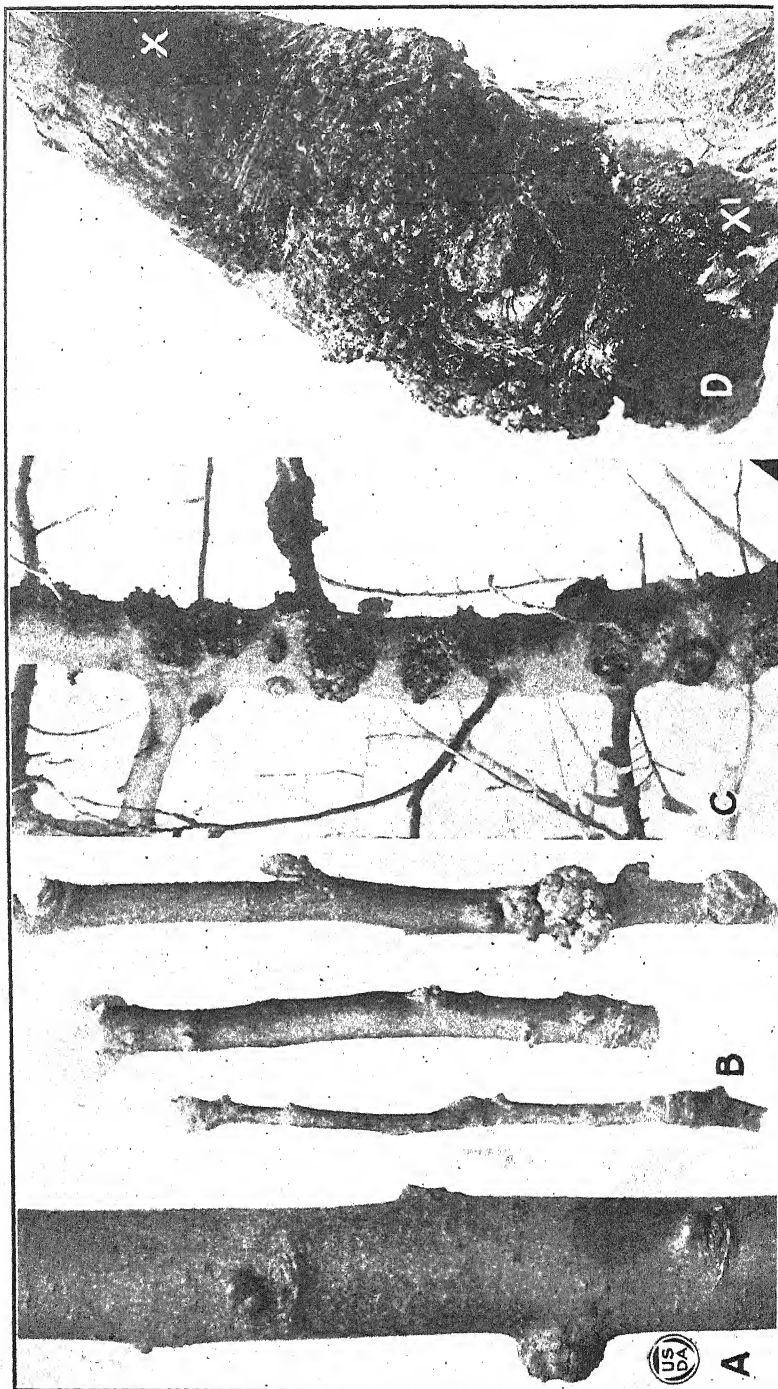


PLATE 2

A.—Early stage of apple stem-tumor on Chenango Strawberry apple tree, West Virginia; natural size.

B.—Young stages of stem-tumor on Buckskin apple tree. The three pieces show three different years' growth of tumors on the same stem. Natural size. Plated from the largest outgrowth, but no crown gall organism was present.

C.—Older stage of stem-tumor on Buckskin apple tree, showing masses of aborted roots; one-fifth natural size. Same tree as B.

D.—Old stem-tumor on Spitzenberg apple tree from Oregon showing pear blight at X and X' as a secondary disease. Natural size.

### PLATE 3

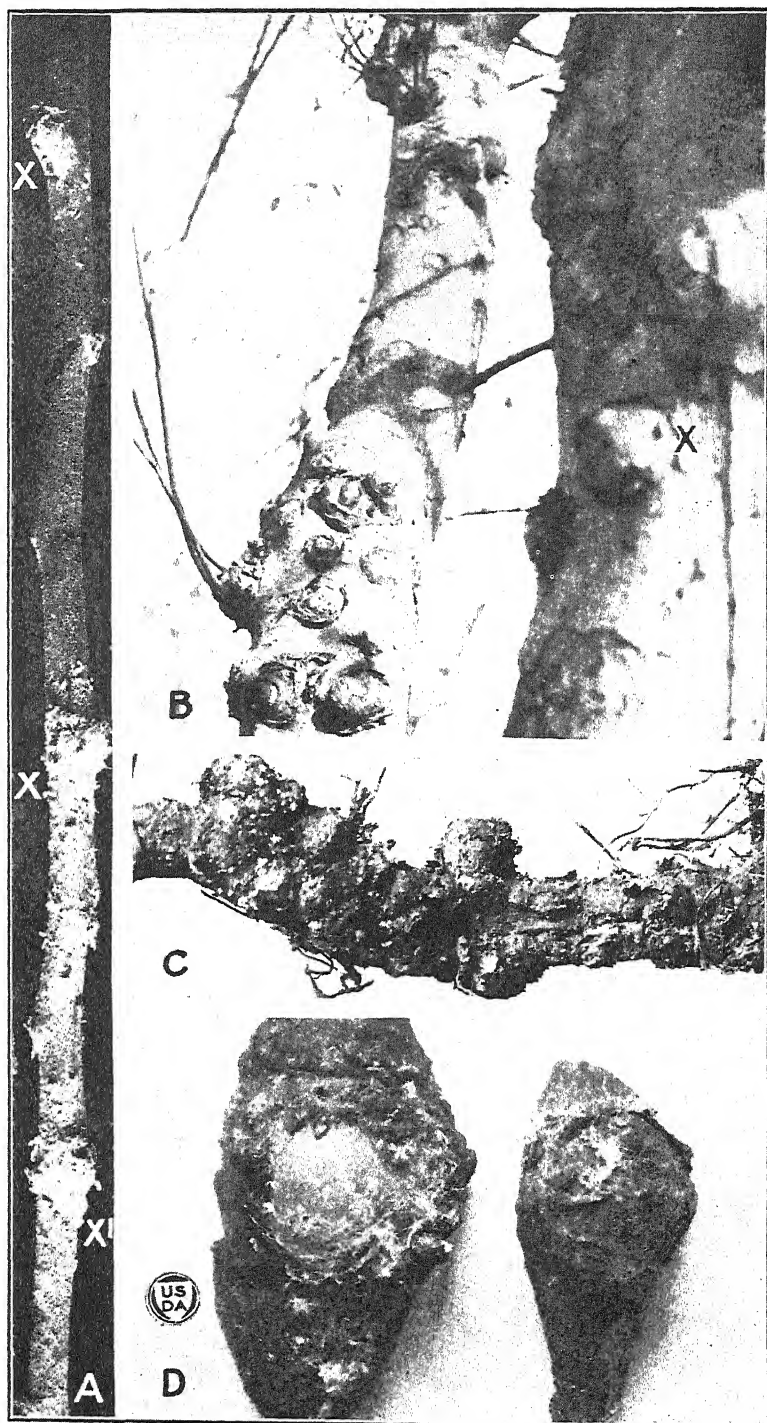
A.—Beginning of apple stem-tumor at X, and X'. Woolly apple aphid abundant at these places. Early Harvest apple tree, Maryland.

B.—Type of stem-tumor on Martin tree, which shows few or no masses of aborted roots. At X a few are ready to burst through the bark. Platings were made from tumor X. No crown gall organism was present. Woolly apple aphids were on these tumors; one-third natural size.

C.—Woolly apple aphid root galls on Buckskin apple tree; natural size. Same tree from which stems B and C of Plate 2 were taken.

D.—Early stage of apple stem-tumor on Buckskin apple tree from Virginia.  $\times 5$ . Negative platings were made from these also. Note presence of woolly apple aphid.







# MORPHOLOGICAL CHARACTERS OF *ALTERNARIA MALI* ROBERTS<sup>1</sup>

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Some years ago *Alternaria mali* was described by the writer (16)<sup>2</sup> and shown to be involved in a peculiar spotting of apple leaves. *Alternaria mali* does not originate these spots, but, gaining entrance through areas killed by *Physalospora cydoniae* (*Sphaeropsis malorum*), by chemicals such as sprays, or by other means, enlarges the dead spots, giving them a peculiar and characteristic appearance. A spot thus formed consists of the original dead area with semicircular or crescent-shaped enlargements about it (Pl. 1, B). One might conceive of such a spot as being formed by infections taking place indiscriminately within the tissues of the original dead area. The fungus growing at equal pace in all directions from each of these infection centers would occupy circular areas, only part of which would project beyond the original spot. Where there is a single infection, in the center of a circular spot, the enlargement appears as a differently colored zone surrounding the original spot and concentric with it. By means of new infections the increase in size may be such that one spot may involve almost an entire leaf, appearing as a more or less circular (original) spot surrounded by consecutive crescent-shaped enlargements. The striations or zones within the spots greatly resemble those produced by species of *Alternaria* on the leaves of other hosts.

Crabill (7) does not agree that *Alternaria mali* is the cause of the enlargement of spots initiated by *Physalospora cydoniae*. His inoculation experiments were apparently very few in number and his results indecisive. Of the writer's work Crabill states: "His photographs, however, show that the enlargements produced by artificial inoculation with *A. mali* are not at all typical. They are too uniformly spreading to resemble the clear-cut crescents of the typical frog-eye spot." Yet these photographs show a remarkable resemblance to figure 4 of his bulletin, labeled "Frog-eye leaf spots as they appear in midsummer." As a matter of fact, both Crabill's photographs and those of the writer would have shown the crescents more clearly cut had the leaves been allowed to become older and dry before photographing. The writer's experiments involved more than 3,000 spots, and the results in all experiments involving *Alternaria mali* were quite decisive. The fungus was found constantly associated with characteristic spots on apple leaves from Virginia, Maryland, Tennessee, Arkansas, and Missouri.

## THE GENUS *ALTERNARIA*

The difficulties underlying attempts to identify species of *Alternaria* are appreciated by all those who have ever undertaken the task. Many

<sup>1</sup> Received for publication February 20, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 707-708.

of the species are described so vaguely or so briefly that almost any member of the genus could be placed within them. Many investigators, finding an *Alternaria* on a new host, have made a new species of it and published a brief description wholly inadequate for purposes of positive identification.

Elliott (9) has done much toward the establishment of the generic limitations of *Alternaria* and has sorted out some of the species into groups. He states: "No final disposition of the present specific names of *Alternaria* and *Macrosporium* can be made without a study of authentic specimens of each species. Most of the descriptions are far from being complete or definite enough to permit their being used for this purpose." Until such work as Elliott suggests is done, the identification of species of *Alternaria* must in most cases be quite uncertain. \*

Elliott's emended description of the genus is such that *A. mali* undoubtedly belongs within it. His description is as follows: "*Alternaria* Nees. Conidiophores solitary or fasciculate, erect or subdecumbent, simple or branched, generally short, colored. Conidia muriform, often with few longitudinal septa, ovate, obclavate, or elongate, always with more or less definitely pointed apex, often long-beaked, colored, under favorable conditions forming chains. (Ex. *A. tenuis*, type of the genus.)"

The writer has studied *Alternaria mali* as found growing in dead spots on apple leaves and as cultivated on artificial media. In addition, *Alternaria tenuis*, from Europe, and species isolated from the leaves of lilac, forsythia, and blackberry and from the fruits of apple, cranberry, and blueberry have been grown on artificial media and studied.

All these forms, while resembling one another in many respects, revealed many points of difference. In some species the hyphae were almost colorless, forming a thin scarcely perceptible crust over the surface of a plate of corn-meal agar with an exceedingly scant production of conidia and no aerial hyphae. Others formed a dense, nearly black crust with a copious production of conidia and no aerial hyphae. Still others produced a black crust with abundant conidial production at its surface and an abundance of more or less flocculent aerial mycelium or a greenish-gray aerial mycelium so dense as to form a thick carpetlike growth over the surface of the culture medium. A few produced an abundance of dirty white aerial hyphae with a very scant growth in the medium or along its surface and a very scant production of conidia.

In the species studied the conidia conformed to the generic description, that is, they were ovate, obclavate, or elongate. In most of these obclavate conidia predominated, but ovate conidia were found in cultures of all except one form which produced elongate forms exclusively. In the species producing practically no aerial hyphae, elongate conidia predominated. While the surfaces of the spores were usually smooth, in some forms verrucosity was quite common. An entire conidium or only a part of it often possessed a verrucose surface. If only a part of the conidial wall was verrucose, it was in a strip or band at right angles to the long axis of the conidium. Verrucosity or absence of verrucosity was not so much a difference between conidia as between chains of conidia, the conidia of individual chains usually being all verrucose or all smooth-walled. The presence or absence of verrucosity appears to be due to highly localized conditions of the immediate environment, if one may judge from selection experiments reported upon later in this paper.

The conidia of all the species were produced in chains with the narrower end distal. De Bary (1), Jones (11), Elliott (9), and many others found

this to be true of the species studied by them, and that the conidia of all species of *Alternaria* are so arranged is generally believed. However, in examining published illustrations of *Alternaria*, even of quite recent date, one finds drawings in which the broader end of the conidium occupies the distal position. It would be interesting to know whether or not such forms really exist.

At the distal end of each conidium, except quite frequently the last one formed in the chain, there is a stalklike projection, variously known as the isthmus, beak, or appendage. It is not so deeply colored as the conidium and may even be hyaline or nearly so. It may be quite short and swollen or long and slender, nonseptate, uniseptate, or even multi-septate (fig. 1). It is formed by a growth or a budding of the conidium at a time when it is the youngest of the chain, that is, when it occupies the distal position in the chain. From this outgrowth the next spore in the chain is developed. In descriptions of species of *Alternaria* the term conidiophore is usually applied only to the outgrowth from the conidia-bearing hypha which supports the first formed conidium of the chain. In this sense it is considered as the conidiophore for the whole chain. The so-called isthmus or beak may also be considered as a conidiophore, not in relation to the conidium from which it is an outgrowth and to which it remains attached, but in relation to the distally situated conidium which grew out from it. This view is supported by the fact that it becomes separated from this conidium as most conidiophores become separated from the conidia which they bear. In one form studied the so-called isthmi or beaks tended finally to collapse and disintegrate, which is also a fairly common behavior of conidiophores. A consideration of isthmi as conidiophores also offers an explanation as to why the end spores of the chains often have no isthmi or have especially long ones. In the former case budding has not started; in the latter the long, septate hyphalike structures which may become new conidia and conidiophores have been formed. The same sort of budding, but at right angles, makes the branches of the chain. The isthmi or conidiophores often do not have as thick walls as the conidia. The tip often appears to be swollen and turgid, a condition which facilitates the breaking up of the chain through lessening the line of contact between the isthmus and the immediately distal conidium. When, however, a breaking up of the chain does not occur at this stage, as often happens when the chain lies along the surface of the culture medium, it is finally brought about by the shrinkage and disintegration of the isthmi. In this way, also, they perform a separative function. They are often more than one-celled and are incapable of germination. In the case of mature conidia the dividing line between conidium and isthmus (conidiophore) is easily determined by the position of the convex end of the conidium and by the greater width and deeper color of the conidium; in older conidia the disintegration of the isthmus may also indicate the dividing line.

#### TECHNICAL DESCRIPTION

*Alternaria mali*.—Realizing that the original description of *Alternaria mali* (16, p. 58) was too brief to be of proper use in identification, the writer undertook a more careful study of the fungus as it develops naturally on apple leaves, as it appears on apple leaves left in moist chambers for several days, and as it grows on artificial culture media. It is hoped

that the description which follows is sufficiently detailed to permit of identification by subsequent investigators, though lack of knowledge concerning the genus as a whole prevents one from knowing what characters should receive particular attention.

Hyphae on the surface of apple leaves are normally scant or lacking, dark olive when present; but in moist chambers they are abundant and

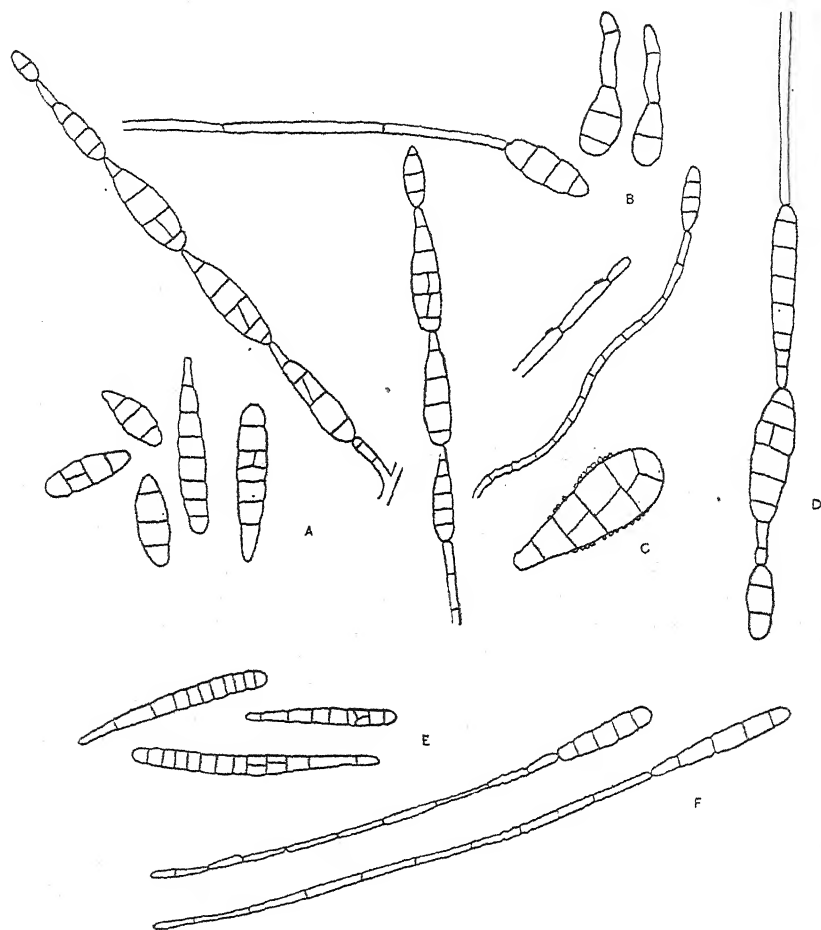


FIG. 1.—A. Conidia of *Alternaria mali* as produced on apple leaves and above the surface in corn-meal agar cultures. B. Types of conidiophores including the so-called beaks or isthmi. C. Outline of a verrucose conidium. D. Conidia produced on the surface of the medium in corn-meal agar cultures. E. Conidia of form B produced at the surface of or in the culture medium. F. Conidia of form B with long so-called beaks or isthmi. A, B, D, E, and F  $\times 325$ . C  $\times 525$ .

are light gray in mass with occasional darker areas. In corn-meal agar cultures the hyphae at or near the surface of the medium vary from nearly hyaline to dark olive, the latter predominating. On apple leaves their arrangement is usually fasciculate, but on artificial media they may lie parallel to one another and nearly in one plane, forming a sheet or layer. The hyphal segments are short, often no longer than their width, without constrictions at the septa. They are 3 to 8  $\mu$  wide and seldom

more than twice as long, mostly unbranched except that conidiophores may spring from them. Aerial hyphae either on apple leaves kept in moist chambers or in corn-meal agar cultures are nearly hyaline to light amber, often geniculate, branched, occasionally joined together (reticulate), segments relatively much longer than in surface hyphae 3 to 5  $\mu$  wide, length quite variable 5 to 25  $\mu$  or longer, often constricted at the septa, especially when old.

In old cultures the hyphal segments often become swollen to form chains of chlamydosporelike bodies.

The conidia (fig. 1 and Pl. 1, C) on apple leaves form tiny black masses, which separate easily from the leaf. In corn-meal agar cultures, chains of conidia form a dark carpet-like mass over the surface of the medium. Conidia also occur to some extent scattered through the aerial hyphae and at the surface of or in the medium. They are light amber to very dark olive or nearly black, those produced just above the surface of the medium being the darkest and those produced on aerial hyphae well above the medium being the lightest. The typical arrangement is in simple or branched chains. Septation may be transverse and longitudinal (muriform), with constriction at the septa especially when old. Verrucose outer walls (fig. 1, C) are common and sometimes the rule. The number of septa vary with the size and shape of the conidium. In general, conidia produced along the surface of or in the artificial culture medium are much longer and narrower than those produced in chains projecting above the medium or on aerial hyphae (fig. 1, D, E). Narrow conidia are more often without longitudinal septa.

The conidiophore subtending the first-formed conidium of a chain is produced at approximately right angles to the hypha (fig. 1, A). It is often short and nonseptate or one-septate, but it may be long and multiseptate (fig. 1, B). Usually, it is broader than the hypha from which it was produced. When the conidium separates from it, a dark-colored scar is seen at the point of attachment (fig. 1, B). It is usually smooth with a swollen apex, but in older cultures it may be at least as many as three times geniculate, each joint denoting the origin of a chain of spores (fig. 1, B). These conidiophores of the initial spores of the chains are usually fasciculate on apple leaves in nature, and are often so in artificial cultures.

The conidiophores (isthmi) supporting subsequent members of the chain are outgrowths from terminal (distal) segments of conidia, or in branched chains, lateral outgrowths from nonterminal segments. They resemble the conidiophore of the initial conidium very closely. They are swollen at the tip, may be nonseptate or multiseptate, short or long, straight, angled, or geniculate (fig. 1, B). In color they range from nearly hyaline to olive, the former predominating except when old.

In all the following measurements and in all consideration of septation the conidiophore or isthmus is not regarded as part of the conidium. Unless otherwise specified the word "septate" means "transversely septate." In each series, the measurements (in microns) are of 50 conidia taken at random. Conidiophores were measured at their greatest width.

Measurements of conidia from spots on apple leaves. (Arlington Experiment Farm, Rosslyn, Va., September, 1921):

	Average.
1-septate (2 per cent), 16 x 9.....	16 x 9
2-septate (10 per cent), 19 to 22 x 8 to 13.....	21 x 11
3-septate (56 per cent), 20 to 29 x 8 to 13.....	25 x 12
4-septate (22 per cent), 27 to 38 x 9 to 15.....	32 x 13
5-septate (10 per cent), 42 to 46 x 9 to 15.....	43 x 13
With longitudinal septation (78 per cent) width 11 to 14.....	13
Without longitudinal septation (22 per cent) width 8 to 13.....	10
Conidiophores (isthmi), 2 to 30 x 3 to 5.....	6 x 4
Number of septa for all the conidia.....	3.28
Dimensions of all the conidia.....	28 x 12

Measurements of conidia from a nine-day-old culture on a corn-meal agar plate (fungus newly isolated from spots on apple leaves):

	Average.
1-septate (2 per cent), 13 x 7.....	13 x 7
2-septate (18 per cent), 17 to 21 x 8 to 13.....	19 x 11
3-septate (46 per cent), 21 to 29 x 9 to 13.....	24 x 11
4-septate (22 per cent), 29 to 37 x 9 to 14.....	34 x 12
5-septate (6 per cent), 34 to 38 x 8 to 13.....	36 x 11
6-septate (6 per cent), 36 to 46 x 10 to 13.....	41 x 12
With longitudinal septation (76 per cent), width 10 to 14.....	12
Without longitudinal septation (24 per cent), width 7 to 11.....	9
Conidiophores (isthmi) 2 to 12 x 2 to 5.....	5 x 4
Number of septa for all the conidia.....	3.30
Dimensions of all the conidia.....	27 x 11

Measurements of conidia from a nine-day-old culture on a corn-meal agar plate (fungus isolated from apple leaves and grown in artificial culture about one year):

	Average.
1-septate (8 per cent), 13 to 15 x 8 to 9.....	14 x 8
2-septate (20 per cent), 17 to 25 x 8 to 13.....	20 x 10
3-septate (56 per cent), 20 to 29 x 8 to 13.....	25 x 11
4-septate (8 per cent), 25 to 34 x 9 to 12.....	30 x 11
5-septate (4 per cent), 34 x 11 to 12.....	34 x 12
6-septate (4 per cent), 42 x 9 to 13.....	42 x 11
With longitudinal septation, 58 per cent, width 9 to 13.....	12
Without longitudinal septation, 42 per cent, width 8 to 11.....	9
Conidiophores (isthmi), 3 to 12 x 3 to 5.....	5 x 4
Number of septa for all the conidia.....	2.92
Dimensions of all the conidia.....	24 x 11

*Alternaria mali*, in addition to causing enlargements of dead spots on apple leaves, is capable of infecting the fruit. It is reported by Reed and Crabill (15) as causing a soft rot of the fruit of the York Imperial variety of apple following "skin crack" and "York spot."

Wolf (21), Longyear (13), Clinton (5), Stakman and Rose (19), and Cook and Martin (6) reported on species of *Alternaria* isolated from apple fruits, but gave no descriptions of them. Longyear's drawings show that his fungus greatly resembles and is possibly identical with *Alternaria mali*. Longyear also found the fungus growing on the leaves and sprouts of the pear.

McInnes (14) and Horne (10) give descriptions of the species of *Alternaria* which they found in dead spots on apple fruits. Of these species, *Alternaria pomicola* Horne has much larger spores than *Alternaria mali*. The *Alternaria tenuis* variety X of McInnes resembles *Alternaria mali* much more closely but has somewhat larger conidia.



From zonate spots on leaves of the common lilac growing near the apple trees at Arlington Experiment Farm, from which *Alternaria mali* was obtained, an *Alternaria* was isolated whose conidial measurements closely approximate those of *Alternaria mali*. In corn-meal agar cultures, however, it forms a thick olive green mat over the medium which is quite different from any observed growth of *Alternaria mali*.

#### VARIATIONS IN *ALTERNARIA MALI*

Variations, mutations, and races or strains among species of the "Fungi Imperfecti" have been noted by Bonar (2), Brierley (3), Burger (4), Crabill (8), La Rue (12), Shear and Wood (18), Stevens (20), and others. The observation that many of the conidia of *Alternaria mali* have verrucose walls induced the writer to attempt to secure by selection a race having smooth conidia exclusively and another having verrucose conidia only. Starting with the progeny of a single conidium, selections of nonverrucose conidia were made through one series, while in another series attempts were made to select those having the highest degree of verrucosity. The method of isolating the selected spores has been described by the writer (17) in a previous publication. It was thought that continued selection might bring success, but after 22 generations, covering a period of two years, no difference in verrucosity could be noted between the conidia of the two series. The results of these selections agree with those obtained by La Rue (12) in his selections for spore length and length of spore appendages in *Pestalozzia guepini*. It is possible that environment is the sole reason for the variation in verrucosity, but the difficulties surrounding such attempts at selection are very great and no conclusion should be drawn. One can not be sure that out of all the conidia produced in the selected culture he is choosing those showing the greatest verrucosity. Neither can he be certain that the conidium which he considers as smooth might not become verrucose when older. Thus he may be simply selecting along an average. It must also be remembered that a single conidium of *Alternaria* is really not a single but a composite conidium usually consisting of many parts each capable of independent germination. The chance for variation in the progeny of a single conidium accordingly may be very great.

While carrying on this work the writer observed a plate culture in which one section of the growth appeared quite different from the remainder. In this part, which will be designated as "A," practically no aerial mycelium was produced, but there was a dark carpetlike mass over the surface of the medium, with olivaceous conidia produced in large numbers. The remainder of the growth, which will be designated as "B," had abundant gray aerial mycelium with a scant production of long amber-colored conidia occurring in long chains running along the surface of the medium and parallel to it (fig. 1, E, F). Selections were made from each of these two forms for the purpose of attempting to establish "pure lines." Transfers were made weekly from the growths of previous transfers showing the greatest growth of A and B, respectively. The selections were grown side by side, one of A and one of B on corn-meal agar in the same Petri dish. From the first, selection A showed but little tendency to break up into A and B. After the tenth selection it came true until the cultures were discarded. B broke up into A and B sections with great constancy during the first 57 selections, though the

B parts of the growth were usually much the larger. From the fifty-eighth to the sixty-ninth and last selection B remained constant. (Pl. 1, A, Pl. 2, A, B.)

We thus have two races arising from a single conidium, neither of which in culture is exactly like the parent. A lacks the aerial hyphae of the parent, while B fails to produce the thin carpetlike layer bearing characteristic conidia, producing, instead, the long slender spores at or beneath the surface of the culture medium. B grows more rapidly than A. There was no chance that A and B were from a mixed or contaminated culture, for they were not only the progeny of a single conidium, but of a succession of 15 singly selected conidia.

Brierley (3) suggests that while variations in the "Fungi Imperfecti" may be due to mutation, they may perhaps be more wisely interpreted in terms of the splitting of an originally impure genetic constitution or of gametic or somatic segregation from heterozygotes. In fungi not known to have a sexual stage, he suggests the possibility of genetic contamination by the fusion of hyphal cells. Until such contaminations can be shown to occur, it seems more logical to consider the aberrant strains as due to mutation while admitting the possibility of genetical contamination. The fact that neither of the two races of *Alternaria* was exactly like the parent lends some support to the genetical contamination theory.

The following measurements were obtained from 50 conidia of each race taken at random from nine-day-old cultures growing side by side in a corn-meal agar plate. Conidial measurements are in microns and do not include conidiophores (isthmi). Unless otherwise specified the word "septate" means "transversely septate."

## RACE A

	Average.
1-septate (4 per cent), 9 to 13 x 7 to 8.....	11 x 8
2-septate (26 per cent), 15 to 21 x 7 to 11.....	18 x 8
3-septate (34 per cent), 21 to 28 x 7 to 11.....	23 x 9
4-septate (10 per cent), 27 to 36 x 8 to 12.....	32 x 10
5-septate (18 per cent), 34 to 38 x 8 to 12.....	35 x 9
6-septate (6 per cent), 38 to 42 x 9 to 12.....	39 x 10
7-septate (2 per cent), 42 x 11.....	42 x 11
With longitudinal septation (36 per cent), width 8 to 12.....	10
Without longitudinal septation (64 per cent), width 7 to 11.....	8
Conidiophores (isthmi) 4 to 6 x 3 to 6.....	5 x 4
Number of septa of all the conidia.....	3.38
Dimensions of all the conidia.....	26 x 9

## RACE B

	Average.
3-septate (12 per cent), 27 to 34 x 8 to 12.....	32 x 10
4-septate (18 per cent), 34 to 42 x 8 to 9.....	39 x 9
5-septate (22 per cent), 38 to 57 x 7 to 9.....	45 x 8
6-septate (18 per cent), 40 to 55 x 8 to 12.....	48 x 9
7-septate (20 per cent), 57 to 67 x 7 to 13.....	60 x 10
8-septate (4 per cent), 76 x 9 to 10.....	76 x 10
9-septate (4 per cent), 63 to 80 x 11.....	72 x 11
10-septate (2 per cent), 88 x 10.....	88 x 10
With longitudinal septation (18 per cent), width 10 to 13.....	12
Without longitudinal septation (82 per cent), width 7 to 10.....	9
Conidiophores (isthmi) 5 to 14 x 4 to 6.....	8 x 5
Number of septa for all the conidia.....	5.54
Dimensions of all the conidia.....	49 x 9

The following measurements of 50 conidia from a 16-day-old culture of race B on corn-meal agar are of interest because of the long conidiophores (isthmi):

	Average.
2-septate (2 per cent), $21 \times 8$ .....	$21 \times 8$
3-septate (10 per cent), $22$ to $39 \times 7$ to $9$ .....	$30 \times 9$
4-septate (18 per cent), $32$ to $42 \times 8$ to $9$ .....	$37 \times 8$
5-septate (30 per cent), $32$ to $52 \times 8$ to $11$ .....	$40 \times 9$
6-septate (22 per cent), $44$ to $52 \times 8$ to $10$ .....	$48 \times 9$
7-septate (14 per cent), $48$ to $59 \times 8$ to $12$ .....	$54 \times 9$
9-septate (4 per cent), $52$ to $63 \times 8$ .....	$58 \times 8$
With longitudinal septation (16 per cent), width $9$ to $12$ .....	$10$
Without longitudinal septation (84 per cent), width $7$ to $9$ .....	$8$
Conidiophores (isthmi) $6$ to $59 \times 3$ to $5$ .....	$18 \times 4$
Conidiophores having a length of more than $20$ were $1$ to $4$ septate.	
Number of septa of all the conidia.....	$5.22$
Dimensions of all the conidia.....	$43 \times 8$

The larger conidia and conidiophores of race B were possibly due to the nutritional advantages incident upon their location in or at the surface of the culture medium. It should be noted that the measurements of race A and the parent are not greatly different. The differences between A and B were not due to differences of environment, since they were grown side by side in the same plate, but it is possible that the conditions attending growth on culture media may have acted as a stimulus to the disclosure of certain differences not apparent in nature.

Mutations were also observed in other single conidium cultures of *Alternaria mali* from apple leaves and single conidium cultures of the *Alternaria* isolated from lilac leaves. On the other hand, species from cranberry and blueberry supplied by Dr. Neil E. Stevens and subsequently grown by the writer from single conidia showed no tendency toward mutation. An *Alternaria* from blackberry supplied by Dr. B. O. Dodge showed two distinct forms from the first culture. Single conidium cultures were not used here, however, and it is possible that two distinct species were present. A culture of *Alternaria tenuis* supplied by Dr. Johanna Westerdijk showed no tendency toward variation, though grown in plate cultures with frequent transfers.

#### SUMMARY

*Alternaria mali* often enters apple leaves through injured or dead spots and forms about them characteristically crescent-shaped or circular enlargements. A brief discussion of some of the morphological characters of the genus *Alternaria* based on observation of species from apple, lilac, cranberry, blueberry, blackberry, and forsythia is given. *A. mali* is described more fully than formerly and more detailed measurements of conidiophores and variously septate conidia are given. Variants, considered as due to mutation, were found in "single spore" cultures of *A. mali*.

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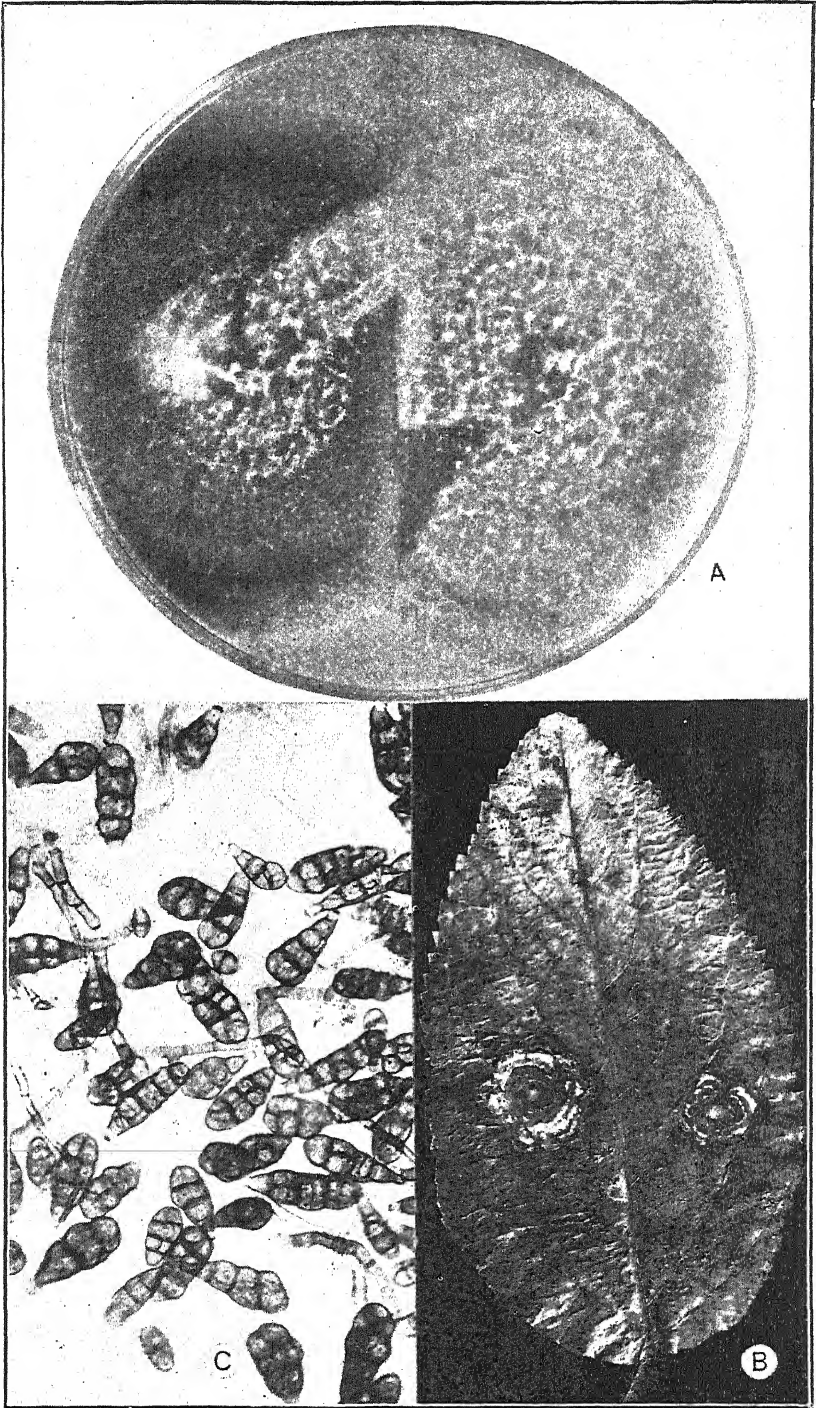


PLATE 1

A.—*Alternaria mali*. Cultures of forms A and B in a Petri dish of corn-meal agar. Each has broken up into the two forms.

B.—Spots on an apple leaf showing about the original central spot the type of enlargements caused by *Alternaria mali*.

C.—Conidia of *Alternaria mali* from a culture on corn-meal agar.  $\times 440$ .



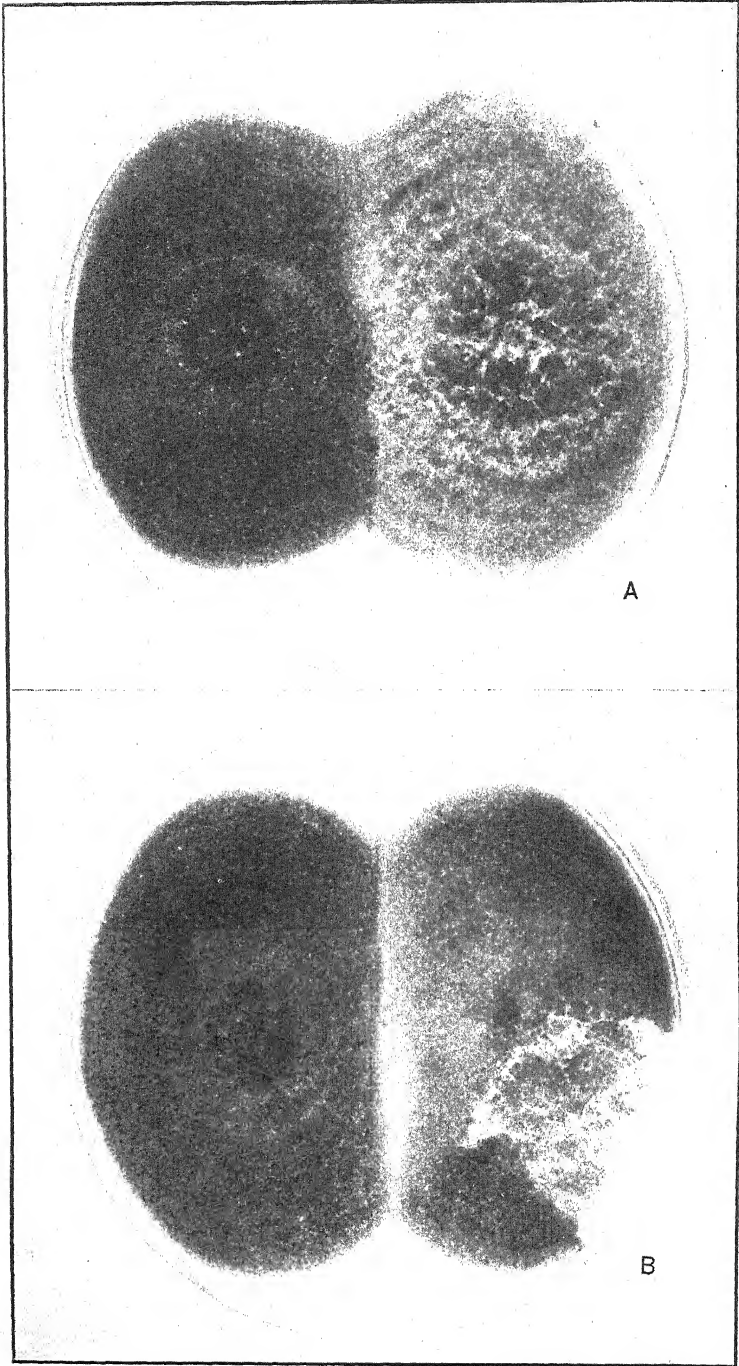




PLATE 2

*Alternaria mali*. Cultures of forms A and B in Petri dishes of corn-meal agar.

A.—Both forms have come true.

B.—Form A has come true but B has broken up, producing much more of A than of B.



# NATURAL ANTISHEEP AMBOCEPTOR AND COMPLEMENT IN THE BLOOD OF FOWLS<sup>1</sup>

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## INTRODUCTION

The attention of the writers was first directed to the dissolving action of fresh chicken serum upon the blood corpuscles of the sheep during some hemolytic experiments with the blood serum of chickens known to be carriers of *Bacterium pullorum*. Whether the lytic action of the chicken serum was due to the presence of a natural antisheep amboceptor or to complement, could not be stated. An endeavor was therefore made to determine the presence of these elements in the serum.

## REVIEW OF LITERATURE

Bordet (2)<sup>2</sup> was the first to call attention to the hemolytic and hemagglutinating action of chicken serum upon the cells of other animals. He also noted the marked susceptibility of rabbit corpuscles to the action of this serum. He was able to produce an antihemolysin that prevented the action of chicken serum upon rabbit corpuscles. In discussing the presence of sensibilisatrice in normal sera he states that it is necessary to be cautious in drawing conclusions. Because a serum agglutinates blood cells does not necessarily mean that they are sensitized to the action of alexin. For example, he states that rabbit corpuscles, although strongly agglutinated by chicken serum heated to 55° C., are then no more susceptible to the lytic action of normal guinea-pig serum than are rabbit corpuscles untreated by heated chicken serum.

Müller (5) verified Bordet's observation, but concluded that the hemolytic action of chicken serum is to be ascribed to an amboceptor-alexin combination. This author did not succeed in separating amboceptor and alexin by use of absorption in the cold as recommended by Ehrlich. Müller sought to demonstrate the presence of the two components by adding an amount of fresh chicken serum that would not hemolyze a given amount of rabbit cells and by the addition of inactivated (heated) serum so to reinforce the hemolytic strength that solution of the blood corpuscles would take place. His results were negative. The negative results were thought to be due to the fact that chicken serum has so little alexin that it could not activate the amboceptor present and would naturally exert no effect. To prove this point he increased the alexin of the chicken serum by injecting the birds with various substances such as peptone, bouillon and aleuronat. By this method the alexin content was increased so that from 0.03 to 0.07 cc. of this fresh serum would cause complete hemolysis of rabbit corpuscles in the presence of 0.2 to 0.3 cc. of heated chicken serum. The fresh serum alone was less active than the fresh serum plus heated serum. The heated

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 715.

serum alone was completely inactive. It was found that about 0.15 cc. of fresh pigeon serum hemolyzed 1 cc. of a 5 per cent suspension of rabbit corpuscles in the presence of 0.2 cc. of inactivated chicken serum.

Lüdke (4) discusses hemagglutinins in chicken and duck serum for the red blood corpuscles of other species. He found that fresh serum from a 3 day-old chick agglutinated and dissolved human and rabbit blood corpuscles in about 10 minutes, but this serum lost the lytic power and most of the agglutinating power after being heated for one-half hour at 55° C.

Serum from a normal 3-day-old duck agglutinated blood corpuscles of the sheep almost immediately, in dilutions of 1 part to 10, and slightly in dilutions of 1 part to 20. The hemagglutinins disappeared when the serum was heated to 60° to 65° C. Fresh duck serum caused complete agglutination of guinea-pig corpuscles in 5 minutes. Such serum showed a trace of agglutination for human corpuscles and a strong agglutination of rabbit corpuscles followed by lysis. After heating this serum at 50° to 55° C. there was partial agglutination of guinea-pig cells, none for human corpuscles and complete agglutination in 5 minutes for those of the rabbit. After heating at 55° to 60° C. no agglutination for guinea pig or human corpuscles was observed, but complete agglutination for rabbit corpuscles occurred in one-half hour.

Rywosch (7) studied the hemolytic action of the blood of embryo chicks upon rabbit corpuscles. No hemolysis could be demonstrated with embryo chick serum. One-tenth cc. of serum from a chick 5 days of age caused complete hemolysis of 0.2 cc. of 5 per cent rabbit cell suspension and was almost complete for 0.5 cc. This author found that 0.08 cc. of serum from an adult bird caused complete hemolysis of 1 cc. of 5 per cent suspension of rabbit corpuscles. An attempt to show that the negative results were due to lack of amboceptor failed. These tests were conducted with varying amounts of chick embryo serum and inactivated serum from adult birds. Serum from 5-day-old chicks added to the inactivated serum from adult birds caused complete hemolysis. This author seemed to think that the embryo serum lacked both amboceptor and complement for rabbit cells.

Sherman (8) examined blood from chick embryos and reported as follows: In no case was lysin found in any embryo except those of 21 days' incubation, and in this case the chicks were pecking their way out of the shell. Their serum contained lysin for rabbit erythrocytes only, 0.1 cc. of serum being required to lase completely 0.1 cc. of a 1 per cent erythrocyte suspension. Complement was found in the embryo serums of 17 and 21 days' incubation; it required 0.05 cc. of the serum of the younger embryo and 0.1 cc. of that of the older to lase completely 0.1 cc. of 1 per cent suspension of rabbit erythrocytes when 1 unit of amboceptor (dog) was added. No embryo serum examined contained complement for sheep, goat, or human erythrocytes.

Rissling (6) studied the hemolytic and hemagglutinating action of the serum of various species upon the blood corpuscles of others. He used fresh blood corpuscles. These were washed twice and used in 1 per cent suspension in salt solution. For agglutination tests the serum was inactivated at 56° C. for one-half hour. The tests were incubated at 37° C. for two hours. Goose serum was found to agglutinate sheep cells when diluted to 1 part to 10. Duck and chicken serum in these dilutions did not agglutinate sheep cells. Chicken serum caused agglutination of corpuscles of various animals in the following dilutions: Human, 1 to 50;

horse, 1 to 50; swine, 1 to 300; rabbit, 1 to 300; guinea pig, 1 to 20; goose, 1 to 80; duck, 1 to 90; and pigeon, 1 to 90.

In his hemolytic experiments Rissling used a 5 per cent suspension of corpuscles. The serum was obtained fresh. Goose, duck, pigeon, and chicken serum caused no hemolysis of sheep cells in amounts of 0.5 cc. Chicken serum was found to cause hemolysis of corpuscles of various animals when used in the following amounts: Human, 0.3 cc.; swine, trace in 0.1 cc., and complete in 0.4 cc. Chicken serum to the amount of 0.5 cc. did not hemolyze corpuscles of horse, ox, sheep, guinea pig, goose, duck, or pigeon.

Aschenheim (1) summarized the results of various authors concerning the lytic action of the serum of various animals upon the blood corpuscles of other animals. In the table incorporated in his article he attempted to show the occurrence of natural amboceptor but failed to demonstrate this for sheep corpuscles in fresh normal chicken serum.

Hyde (3), in studying the natural hemolytic antibody of chicken serum, found that 0.1 cc. of chicken serum consistently caused complete hemolysis of 0.1 cc. of a 1 to 4 suspension of corpuscles of rabbit, guinea pig, ox, sheep, and pigeon. The dissolving action, however, was most energetic for rabbit cells and least for those of the pigeon.

Hyde conducted some experiments on the reactivation of heated chicken serum. He was unable to do this by the usual procedure, but by a modification of the original technic, chicken serum inactivated by heat at 56° C. for 30 minutes, was reactivated for rabbit corpuscles with nonhemolytic doses of fresh chicken serum to practically its original hemolytic titer. The test was made by adding the different amounts of heated chicken serum to the tubes, salt solution was then added, followed by fresh chicken serum as a complement. The tubes were incubated for one hour, the corpuscles were then added and the incubation continued. It was found that chicken serum inactivated at temperatures between 53° and 58° C. could be reactivated almost to its original titer by this method. Somewhat similar results were obtained by use of guinea-pig serum as a complement.

#### EXPERIMENTAL DATA

A flock of 22 fowls was available for this study. All birds were bled from the heart. The samples of blood thus obtained were allowed to clot and the serum was removed to sterile tubes immediately after centrifugation.

#### SEPARATION OF AMBOCEPTOR AND COMPLEMENT

In order to make titrations of the amboceptor and complement separately, it was necessary to absorb the former with washed sheep cells at 0° C. The various samples of serum were placed in brine at a temperature of 0° C. A sheep-cell suspension was likewise placed in the bath. After sufficient time had elapsed to allow the temperature of the serum and cells to be lowered to 0° C., 3 cc. of the 5 per cent suspension of cells were added to 1 cc. of each sample of serum. The tubes were thoroughly shaken and immediately replaced in the bath where they were allowed to remain for one hour, which proved to be sufficient time for the absorption of hemolysin by the cells. Each sample was centrifuged and the supernatant fluid removed to sterile tubes. The sediment was resuspended in

a large quantity of cold saline and again thrown down in the centrifuge. After removing the supernatant fluid the sediment was resuspended in 4 cc. of saline.

#### TREATMENT OF SUPERNATANT FLUID

The supernatant fluid containing the complement represented a dilution of one in four. Gradually increasing quantities of this dilution were placed in a series of five tubes and to this was added one unit of anti-sheep hemolysin (rabbit) and 0.5 cc. of a 5 per cent suspension of sheep cells. Saline was added to make the total volume in each tube equal to 2 cc. A control tube was added which contained 0.5 cc. of diluted complement and sheep cells, to determine the presence of amboceptor. Table I gives the amount of each reagent used.

TABLE I.—*Titration of complement in chicken serum*

Tube No.	1	2	3	4	5	6
Supernatant fluid (complement).....	0.2	0.4	0.6	0.8	1.0	0.5
Amboceptor (1 unit).....	.1	.1	.1	.1	.1	.0
Cells (5 per cent).....	.5	.5	.5	.5	.5	.5
Saline.....	1.2	1.0	.8	.6	.4	1.0

All tubes were incubated at 37° C. for one hour and placed in the ice box over night. The results are recorded in Table II.

TABLE II.—*The action of supernatant fluid (complement) and one unit of anti-sheep amboceptor (rabbit) on sheep cells*

	One unit of anti-sheep amboceptor (rabbit) and 0.5 cc. of 5 per cent sheep cells plus supernatant fluid.					0.5 per cent of sheep cells plus supernatant fluid.
Supernatant fluid..	0.2	0.4	0.6	0.8	1.0	0.5
Bird No.						
1.....	0	0	0	0	0+	0
2.....	0	0	0	0	0	0
3.....	0	0	0	0	0	0
4.....	0	0+	+	+	+	0
5.....	0	+	+	+	+	0
6.....	0	0	0	0	0	0
7.....	0	0	0	0	0	0
8.....	0	+	+	+	0+	0
9.....	0	0	0	+	+	0
10.....	0	0	+	+	+	0
11.....	0	0	+	+	+	0
12.....	+	+	+	+	0+	0
13.....	0	0	0	0	0	0
14.....	0	0	0	0	+	0
15.....	0	+	+	+	+	0
16.....	0	+	+	+	+	0
17.....	0	0	0	0	+	0
18.....	0	0	0	0	0	0
19.....	0	0	0	0	0	0
20.....	0	0	0	0	0	0
21.....	0	0	0	0	0	0
22.....	0	0	0	0	0	0

++++=complete; +++=marked; ++=partial; +=trace; 0=no hemolysis.

Examination of Table II shows that there is a complement in some chicken sera capable of activating an antisheep hemolysin (rabbit), when the ingredients are added in the usual order. However, there is considerable variation in the complement content of the various samples of sera tested.

#### TREATMENT OF SEDIMENT

One-half cubic centimeter of resuspended sediment was added to each of a series of 6 tubes. To the first 5 were added gradually increasing quantities of complement (guinea-pig serum diluted 1 to 10). Two-tenths cubic centimeters of this dilution of guinea-pig serum represented one unit of complement. The sixth tube served as a control to detect the presence of complement in the sediment suspension. Saline was added to each tube to make the total volume equal 2 cc. Table III gives the amounts of the various elements used.

All tubes were thoroughly shaken and placed in the incubator at 37° C. for one hour and then placed in the refrigerator over night. Table IV gives the results of each test.

TABLE III.—*Titration of amboceptor in chicken serum*

Tube No.	1	2	3	4	5	6
Suspended sediment.....	0.5	0.5	0.5	0.5	0.5	0.5
Complement (1-10).....	.2	.4	.6	.8	1.0	.0
Saline.....	1.3	1.1	.9	.7	.5	1.5

TABLE IV.—*The hemolytic action of gradually increasing quantities of guinea-pig serum on resuspended sediment*

Complement (1-10).	0.5 cc. of resuspended sediment plus guinea-pig complement.					Control.
	0.2	0.4	0.6	0.8	1.0	0
Bird No.						
1.....	0	0	0	0	+	0
2.....	0	0	0	0	+	0
3.....	0	0	0	0	+	0
4.....	0	0	0	0	+	0
5.....	0	0	0	0	+	0
6.....	0	0	0	0	+	0
7.....	0	0	0	0	+	0
8.....	0	0	0	0	+	0
9.....	0	0	0	0	+	0
10.....	0	0	0	0	+	0
11.....	0	0	0	0	+	0
12.....	0	0	0	0	+	0
13.....	0	0	0	0	+	0
14.....	0	0	0	0	+	0
15.....	0	0	0	0	+	0
16.....	0	0	0	0	+	0
17.....	0	0	0	0	+	0
18.....	0	0	0	0	+	0
19.....	0	0	0	0	+	0
20.....	0	0	0	0	+	0
21.....	0	0	0	0	+	0
22.....	0	0	0	0	+	0

+ = trace; 0 = no hemolysis.

An examination of Table IV shows that, under the conditions of the test, there is very little natural antisheep hemolysin in chicken serum that is capable of being activated by guinea-pig complement. No hemolysis occurred in any tube containing less than 1 cc. of diluted complement, and the degree of hemolysis in these tubes appeared to be the same in all cases. A preliminary titration showed that 1 cc. of a 1 to 10 dilution of this pooled guinea-pig serum would not cause hemolysis of 0.5 cc. of a 5 per cent suspension of sheep cells.

Assuming the possibility that the natural antisheep hemolysin might be better activated by the native complement in chicken serum, it was decided to make such a test on each sample as indicated in Table IV.

The tubes were thoroughly shaken and placed in the incubator at 37° C. for one hour and placed in the refrigerator overnight. Table V gives the results obtained.

TABLE IV.—The hemolytic action of fresh unheated chicken serum on sheep cells

Tube.	1	2	3	4	5
Serum (undiluted).....	0.1	0.2	0.3	0.4	0.5
Cells (5 per cent).....	.5	.5	.5	.5	.5
Saline.....	1.4	1.3	1.2	1.1	1.0

TABLE V.—The hemolytic and hemagglutinating action of fresh unheated chicken serum on sheep cells

Bird No.	0.5 cc. of sheep cells plus serum.					Smallest quantity of serum which caused agglutination of blood cells.
	1-20	1-10	1-6.6	1-5	1-4	
	0.1 cc.	0.2 cc.	0.3 cc.	0.4 cc.	0.5 cc.	
1.....	a o	o	o	o	+	Cc. 0.4
2.....	o	o	+	+	+	.1
3.....	o	o	+	+	+	.2
4.....	o	+	a++	++	++	.2
5.....	o	o	o	+	+	.2
6.....	o	o	o	+	o	.3
7.....	o	o	o	o	o	.5
8.....	o	a+++	+++	+++	+++	.1
9.....	o	+	++	++	++	.2
10.....	o	+	+	+	+	.2
11.....	o	o	+	+	+	.2
12.....	o	+	+	+	+	.2
13.....	o	o	o	+	+	.2
14.....	o	o	o	+	+	.1
15.....	o	o	+	+	+	.3
16.....	o	o	++	+	+	.2
17.....	o	o	+	+	+	.2
18.....	o	o	+	++	+	.3
19.....	o	o	+	++	+	.2
20.....	o	o	o	o	+	b .2
21.....	o	o	o	+	+	.2
22.....	o	a+	++	++	++	.2

a+++ = marked; ++ = partial; + = trace; o = no hemolysis.

b Very slight.



In all sera tested some agglutination of the red cells took place. The smallest quantity of serum causing agglutination is indicated in Table V. It will be seen that some hemolysis occurred in every case except with the serum of fowl No. 7. Variation in the degree of hemolysis is evident; in general the complement titer corresponds in a measure to the hemolytic titer of the fresh serum. The hemagglutinin does not appear to correspond in strength to the complement titer. Nor do the hemagglutinins correspond in strength to the hemolytic action of the fresh serum.

#### CONCLUSIONS

Most samples of fresh chicken serum are capable of causing some hemolysis of sheep cells. The small quantity of natural antisheep amboceptor contained in normal chicken serum may be separated from the complement by absorption with sheep cells at 0° C. The quantity of hemolysin does not vary appreciably in different individuals. The quantity of complement is small as compared with that contained in guinea-pig serum. A variation in the quantity of complement existed in the samples tested. Hemagglutinins for sheep cells are present in fowl sera but the quantity is not in proportion to the degree of hemolysis.

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## EFFECT OF NITRATE APPLICATIONS UPON THE HYDROCYANIC-ACID CONTENT OF SORGHUM<sup>1</sup>

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### INTRODUCTION

It is now generally recognized that growing plants of the sorghum family contain hydrocyanic acid, which is present largely as a glucoside (dhurrin), from which hydrocyanic acid is set free by the action of an enzym normally present in the sorghum plant. Under conditions favorable to the action of this enzym all the hydrocyanic acid is liberated, the time required varying from less than 2 hours at 45° C., to less than 20 hours at room temperature (20° C.).

The proportion of hydrocyanic acid varies much, young plants generally containing a higher percentage than more mature ones grown under like conditions. In green plants the leaves contain a higher percentage than the stems, a fact which may explain part of the difference found between young and old plants, as the leaves of the former constitute a much greater proportion of the total weight of the plant.

In 1903 Brünnich,<sup>2</sup> in Australia, found that sorghum plants fertilized with sodium nitrate contained more hydrocyanic acid than those grown on unfertilized soil.

Four years later Alway and Trumbull,<sup>3</sup> in Nebraska, from the analysis of large, dark green, and of small, yellowish green sorghum plants growing as volunteers, concluded that the higher percentage of prussic acid found in the larger and greener plants was due to more available nitrogen in the soil where they grew.

In 1915 Willaman and West<sup>4</sup> concluded from field experiments in Minnesota that "on soils deficient in nitrogen, added nitrogen may slightly increase the prussic acid in sorghum," while "with a fertile soil and abundant nitrogen this effect may not be produced,"<sup>5</sup> and from later studies<sup>6</sup> that, "unhealthy plants usually contain more hydrocyanic acid than healthy ones. The unhealthy condition may be due to malnutrition, to improper transpiration, to insect pests, or to other causes," while "adequate water supply is usually accompanied by low, and inadequate by high, hydrocyanic-acid content." Vinall<sup>7</sup> from a critical

<sup>1</sup> Received for publication Jan. 11, 1924. Published with the approval of the Director as Paper No. 435 of the Journal Series of the Minnesota Agricultural Experiment Station.

<sup>2</sup> BRÜNNICH, J. C. HYDROCYANIC ACID IN FODDER PLANTS. *In Jour. Chem. Soc. [London]*, v. 83, pt. 2, p. 788-796. 1903.

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<sup>5</sup> ——— OP. CIT., p. 184.

<sup>6</sup> ——— THE EFFECT OF CLIMATIC FACTORS ON THE HYDROCYANIC-ACID CONTENT OF SORGHUM. *In Jour. Agr. Research*, v. 6, p. 271-272. 1916.

<sup>7</sup> VINALL, H. N. A STUDY OF THE LITERATURE CONCERNING POISONING OF CATTLE BY THE PRUSSIC ACID IN SORGHUM, SUDAN GRASS, AND JOHNSON GRASS. *In Jour. Amer. Soc. Agtron.*, v. 13, p. 267-280. 1921.

survey of the literature concludes that injury to growing sorghum plants by frost or drought increases the prussic-acid content, but that stunted growth from lack of plant food in the soil diminishes it.

In view of these divergent conclusions as to the effect of available nitrogen upon the hydrocyanic-acid content, and hence as to the possibility of using sorghum as an indicator in studies of the availability of nitrogen, the writer decided to try the effect of varying amounts of nitrate upon plants otherwise subjected to the same conditions.

## EXPERIMENTAL DATA

### SOILS USED

The plants analyzed were grown in the greenhouse in stoneware jars 8.3 inches in diameter and 8.5 inches deep. Each of the three Minnesota soils selected for use in the experiment were treated alike except in the amount of fertilizers applied. The soils were all low in nitrogen, so that it was to be expected that the addition of nitrogen would produce a marked visible effect on the plants. They were:

Thurston loam (subsoil), from the farm of the Minnesota Agricultural Experiment Station (nitrogen 0.039 per cent).

Merrimac loamy sand, from Coon Creek Sand Experimental Fields (nitrogen 0.068 per cent).

Coloma sand, from Nickerson, in Carlton County (nitrogen 0.068 per cent).

### TREATMENT OF SOILS

Before the Thurston loam was placed in the jars, muriate of potash (52 per cent  $K_2O$ ) and superphosphate (45 per cent  $P_2O_5$ ) were mixed into the soil, each at the rate of 2 pounds per ton (0.1 per cent).

This was equivalent to an application of 1 ton per acre of each of the fertilizers, an amount that should remove any question of a lack of either potash or phosphate. Half the jars received no nitrate, while of the other half each jar received 0.25 gm. at each application, this being given in solution at recorded intervals. The plants were very pale in color and made very little growth where liberal amounts of only potash and phosphoric fertilizers were added, but where sodium nitrate in addition was applied the color was dark green and the growth was excellent.

The Merrimac loamy sand is low in nitrogen, but it had just previously been used in the greenhouse for growing alfalfa, and its available nitrogen had thereby been increased. As this soil had been found not to respond to either potash or phosphate with farm crops, neither of these was added. With this soil, in the early stages of growth there was no difference in appearance between the plants that had received nitrate and those that had not, growth and color being good in both, but later, when some of the nitrogen furnished by the alfalfa roots had been used up, a difference appeared.

On the Coloma sand, to which neither potash nor phosphate was supplied, the growth of the sorghum was very poor even where nitrate was added.

### SORGHUM FROM FIRST PLANTING

All the jars of the three soils were planted with seed from the same lot on December 5, 1922. The growth from this planting was slow, due to the short period of sunshine daily in the winter. The plants grown from

this seeding were all used in perfecting the manipulative procedure, and for these there are no quantitative data. Qualitative tests showed that those plants grown with added nitrate contained a higher proportion of hydrocyanic acid.

#### METHOD OF ANALYSIS

The method of analysis thus developed and used with the subsequent plantings was as follows:

The fresh green material was weighed, passed through a food chopper, and placed in a Kjeldahl flask, with not more than 500 cc. of water, using only enough to wash the sorghum out of the chopper into the flask. This mixture was allowed to autolyze for two hours at 45° C., or over night at room temperature, and then, without addition of acid, distilled into dilute sodium hydroxid solution. The basic distillate was then treated with 2 or 3 cc. of a 3 per cent solution of ferrous sulphate, and allowed to stand about 20 minutes, by which time part but not all of the iron was oxidized to the ferric state, and then made acid with dilute sulphuric acid. The blue color developed slowly or quickly, according to the amount of hydrocyanic acid present, and on this account it was found best for the test solution to stand for some hours before being compared in a Campbell-Hurley colorimeter with the standard color prepared from potassium cyanid.

It was found convenient to make the standard equivalent to 10 mgm. of potassium cyanid per 100 cc. of standard solution, and usually about 30 gm. of the green sorghum was used for each determination, but in the case of green leaves, or of very young whole plants, much smaller samples sufficed, while with large, stocky plants, larger samples were necessary. As about 150 cc. of distillate was usually secured, the volume of the test solution was made up to 200 cc. except in cases of samples that contained unusually large amounts of hydrocyanic acid, when the volume was increased.

#### SORGHUM FROM LATER PLANTINGS

As soon as the first crop had been harvested, seed was planted among the roots, on January 30, and the second crop grown without cultivation or addition of any fertilizer except nitrate.

The plants of the second crop on the Thurston loam subsoil, where no nitrate had been added, were short, slender, and very light in color, indicating extreme nitrogen hunger, and did not furnish enough hydrocyanic acid to permit of its quantitative determination, or in some cases even to permit of its detection (Table I). Even the lighter applications of nitrate produced marked improvement in growth and color, and gave readily measurable amounts of hydrocyanic acid. Larger applications of nitrate raised the hydrocyanic-acid content proportionately.

The nitrogen hunger was not marked in the first crop on the Merrimac loamy sand, and even in the second crop it was not extreme. Although the available nitrogen in the unfertilized jars had been diminished by the first crop, there still remained enough to give a growth to the plants of the second crop better than on either of the other soils. In the case of lots E and F, there was no difference in appearance between the plants grown with and without nitrate, but in lot I the plants without nitrate were much lighter in color. In every lot the percentage of hydrocyanic acid was considerably increased by the addition of nitrate (Table II).

TABLE I.—*Sorghum plants grown on Thurston loam subsoil, with nitrate applied at intervals*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per jar.	Per acre.			Per cent.	Per plant.
		<i>Days.</i>	<i>Gm.</i>	<i>Lb.</i>	<i>Cm.</i>	<i>Gm.</i>		<i>Mgm.</i>
A	1	61	None.	None.	16	0.3	None.	None.
A	2	61	1.25	314	51	7.0	0.0082	0.573
A	3	61	1.25	314	40	5.0	.0070	.350
A	4	61	1.50	376	43	6.0	Trace.	Trace.
A	5	61	1.75	440	40	5.0	.0028	.140
A	6	61	3.25	815	53	9.0	.0049	.430
B	7	64	1.25	314	46	4.0	.0012	.048
B	8	64	1.25	314	.....	5.0	.0033	.165
B	9	64	2.00	502	61	10.0	.0098	.980
B	10	64	5.25	1,317	56	9.0	.0439	3.940
C	11	75	None.	None.	13	0.3	None.	None.
C	12	75	1.50	376	40	3.3	.0029	.096
C	13	75	1.50	376	.....	3.3	.0078	.258
C	14	75	1.75	440	33	4.5	.0016	.072
C	15	75	2.25	564	33	3.7	.0094	.330
C	16	75	10.25	2,572	33	4.5	.0082	.370
D	17	86	1.50	376	48	4.0	.0082	.330
D	18	86	1.75	440	56	4.0	.0348	1.390
D	19	86	2.00	502	46	3.0	.0340	1.050
D	20	86	2.50	625	53	4.0	.0353	1.410
D	21	86	15.25	3,827	53	5.5	.1313	7.250

TABLE II.—*Sorghum plants grown on Merrimac loamy sand with and without nitrate applications*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).		Part of plant analyzed.
			Per jar.	Per acre.			Per cent.	Per plant.	
		<i>Days.</i>	<i>Gm.</i>	<i>Lb.</i>	<i>Cm.</i>	<i>Gm.</i>		<i>Mgm.</i>	
E	22	46	None.	None.	.....	10.0	0.0123	1.230	Whole plant.
E	23	46	1.25	310	.....	9.0	.0246	2.214	Do.
F	24	48	None.	None.	.....	.....	.0098	.....	Leaves only.
F	25	48	1.25	310	.....	.....	.0836	.....	Do.
G	26	63	None.	None.	61	7.0	.0234	1.638	Whole plant.
G	27	63	1.50	375	71	13.0	.0314	4.080	Do.
H	28	72	None.	None.	46	3.5	None.	None.	Do.
H	29	72	None.	None.	.....	4.0	Trace.	Trace.	Do.
H	30	72	1.50	375	56	16.0	.0369	5.91	Do.
H	31	72	1.50	375	.....	10.0	.0345	3.45	Do.
I	32	89	None.	None.	56	7.0	.0041	.280	Do.
I	33	89	2.00	502	69	10.0	.1100	11.00	Do.
I	34	89	2.00	502	64	9.0	.0460	4.14	Do.

All the plants grown on the Coloma sand were small, but the difference both in size and percentage of hydrocyanic acid content was noticeably in favor of those treated with nitrate. The total amount of hydrocyanic acid per plant was a hundred times as great in the latter (Table III).

TABLE III.—*Sorghum grown on Coloma sand, with and without nitrate application*

Lot.	Jar number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per jar.	Per acre.			Per cent.	Per plant.
		Days.	Gm.	Lb.	Cm.	Gm.		Mgm.
J	35	69	None.	None.	36	1.0	0.0933	0.033
J	36	69	1.25	314	56	5.5	.0627	3.448
K	37	89	None.	None.	30	1.0	None.	None.
K	38	89	2.00	502	51	4.0	.1360	5.240

In the fourth column of Tables I, II, and III, is given the amount of nitrate added from the beginning of the experiment. As the nitrate was added in successive doses while the plants were growing, the amount present at any time was less than the total reported, since the plants had used some of it, and, further, there may have been some denitrification. The intention was to compare plants grown with scanty, moderate, and abundant supplies of nitrate, and the analyses reported indicate that the hydrocyanic acid increased with the amount of nitrate supplied, up to amounts beyond those usually considered advisable for field crops.

A third planting was made in April, using fresh Thurston loam subsoil in wooden boxes, 1 foot square, and 8 inches deep. Muriate of potash, at the rate of 300 pounds per acre, and treble superphosphate, 600 pounds per acre, were stirred into the surface of the soil, the seed planted on April 2, and the sodium nitrate given in a single application five days after the planting, at which time the plants were about 1 inch high. Each treatment was in duplicate and in some cases in quadruplicate, and the growth and appearance of all the plants with each treatment were very uniform. This series gave the most concordant results of any, probably because of the more favorable time of the year for growth, April 2 to May 20. Both the size of the plants and the percentage of hydrocyanic acid increased throughout the series, but the plants in the boxes that were given 760 pounds of nitrate per acre showed a slightly less thrifty appearance than those with only 570 pounds (Table IV).

TABLE IV.—*Sorghum plants grown on Thurston loam subsoil with various amounts of nitrate, given in a single application*

Lot.	Box number.	Age of plants.	Nitrate applied.		Average height.	Average weight.	Hydrocyanic acid (HCN).	
			Per box.	Per acre.			Per cent.	Per plant.
		Days.	Gm.	Lb.	Cm.	Gm.		Mgm.
L...	39	33	None.	None.	28	1.0	None.	None.
L...	40	33	0.50	47.5	30	1.2	None.	None.
L...	41	33	1.00	95.0	35	2.6	0.0160	0.42
L...	42	33	2.00	190.0	51	4.0	.0330	1.32
L...	43	33	4.00	380.0	48	4.0	.0810	3.24
L...	44	33	6.00	570.0	53	3.0	.0830	2.50
L...	45	33	8.00	760.0	46	4.0	.1200	4.80
M...	46	47	None.	None.	28	1.0	None.	None.
M...	47	47	.50	47.5	41	1.5	None.	None.
M...	48	47	1.00	95.0	41	3.0	.0025	.075
M...	49	47	2.00	190.0	48	4.0	.0037	.148
M...	50	47	4.00	380.0	58	6.0	.0140	.640
M...	51	47	6.00	570.0	66	8.5	.0290	2.460
M...	52	47	8.00	760.0	69	8.5	.0540	4.600

A qualitative test was made, using much smaller samples than those used in the preceding experiments, in order to determine whether a negative qualitative test might have resulted even from plants containing a considerable amount of hydrocyanic acid, because too small a sample had been employed. As 15 or 20 gm. is about the smallest sample that can be conveniently ground through the food chopper, these small samples of 2 or 3 gm. each were crushed in an agate mortar with a pestle. The plants tested were grown on Merrimac Loamy Sand, with nitrate added, and were dark green in color. They were from 20 to 28 cm. in height, and about 1 gm. in weight each. Two plants were used for each sample. In the analysis of all four samples, a satisfactory blue color resulted, about equal to that from 0.2 mgm. of potassium cyanid. It thus seems probable that working with very small plants, provided the sample is representative and thoroughly crushed or ground and adequately autolyzed, the failure of even a small sample (5 gm.) to give good qualitative test should be looked upon as indicating a probable lack of nitrate in the soil.

Although it has been generally recognized that the content of hydrocyanic acid is much greater in the leaves of sorghum than in the stems, it was not deemed safe to rely on analyses of the leaves only, but in the case of larger plants, the leaves and stems were analyzed separately and the hydrocyanic acid in the whole plant computed from these separate analyses. In the case of young small plants, it is difficult to separate leaf and stem; hence in those cases the whole plant was analyzed as one sample.

A few of these separate analyses of leaf and stem are reported in Table V to show that there is no characteristic difference in distribution of the hydrocyanic acid between fertilized and unfertilized plants, at least at the ages taken. The amount increases with the amount of nitrate supplied, and the percentage is usually several times as great in the leaves as in the stem.

If the plant is employed as an indicator, it may be found that analysis of the leaves only will give as correct an indication of the readily available nitrogen in the soil as analysis of the whole plant.

TABLE V.—Distribution of hydrocyanic acid between leaf and stem of the same plant

Sample number.	Age of plant.	Soil.	Nitrate added per jar.	Hydrocyanic acid content.		
				Leaves.	Stem.	Whole plant.
	Days.		Gm.	Per cent.	Per cent.	Per cent.
107	69	Coloma sand.....	0.75	0.1510	0.0221	0.0627
108	71	Merrimac loamy sand....	None.	None.	None.	None.
109	71	.....do.....	0.75	.1111	.0062	.0369
110	92	.....do.....	None.	.0040	Trace.	.....
111	92	.....do.....	1.00	.1150	.0088	.0417
127	88	Thurston loam subsoil...	1.50	.0610	.0100	.0340
129	88	.....do.....	1.25	.0180	Trace.	.0080
134	.....	Coloma sand.....	.....	.2697	.0519	.1360



## SUMMARY

The hydrocyanic acid was determined in sorghum plants grown in the greenhouse, using three Minnesota soils low in nitrogen and adding sodium nitrate in different amounts. The size of the plants, their color and prussic acid content were all affected by the amount of nitrate applied.

In general, the percentage of hydrocyanic acid in the green plants, was in proportion to the nitrate used. The effect on the prussic acid content continued even beyond the point where nitrate ceased to affect the color and size of the plants. In the light-colored sorghum plants, yellow to yellowish green, the percentage of prussic acid was very low, and in some cases none could be detected, while in all darker colored plants it was readily determined, even single plants weighing only 2 gm. giving distinct qualitative evidence. The leaves of the darker-colored plants contained several times as high a percentage of prussic acid as the stems, and the applications of nitrate showed no distinct effect upon its distribution between stem and leaf.

Sorghum gives promise of being useful as an indicator plant in studies of the supply of readily available nitrogen in soils. It responds readily not only by a more rapid growth and darker color but also by an increased hydrocyanic-acid content, which is highest in the young plants. As analysis requires only small samples; these may be had within a few weeks after the seed is planted.



# THE COURSE OF ACIDITY CHANGES DURING THE GROWTH PERIOD OF WHEAT WITH SPECIAL REFERENCE TO STEM-RUST RESISTANCE<sup>1</sup>

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## INTRODUCTION

Acidity measurements of juice expressed from wheat at frequent intervals from the early-seedling to the late-flowering stage have shown the occurrence of an interesting sequence of changes during the development of the plant. As the investigation was initiated for the study of the problem of disease resistance, the data were obtained on a number of varieties representing extremes of resistance and susceptibility to stem rust (*Puccinia graminis tritici* Erikss. and Henn.). The results therefore not only give evidence from a new standpoint of changing metabolic processes during the life of the wheat plant, but also permit conclusions on the question of a relation between acidity and resistance to stem rust.

## PROCEDURE

The plants were grown in the greenhouse, although occasional checks were made with field-grown plants. The six varieties studied were sown, cut, and handled in pairs, each pair consisting of one relatively resistant and one relatively susceptible wheat. These pairs were the same in each of the three series, namely, Kota and Preston, Pentad (D-5) and Marquis, Khapli emmer and Little Club, respectively.

The same methods were followed for expressing and handling the juice and making the acidity determinations as were described in a preceding paper (4).<sup>3</sup> Both hydrogen-ion and titratable-acid concentrations were determined electrometrically. Immediately following the determination of the hydrogen-ion concentration of a sample, which in each case consisted of 10 cc. of undiluted juice, the electrometric titration was made on the same sample by adding N/20 sodium hydroxid solution, 1 cc. at a time, by means of a burette, the tip of which was inserted through the cork of the electrode vessel. The  $P_H$  values calculated from the successive potential differences so obtained were plotted against the volumes of sodium hydroxid required to produce them. From the resulting curves, the volume of alkali required to bring the reaction to  $P_H$  8.3, the turning point of phenolphthalein, was determined and taken to represent the titratable acidity of the sample. Uniform procedure in the handling of each sample, together with the proper reversal of the sequence of determinations, reduced the chance of error to a minimum and insured the validity of comparisons.

<sup>1</sup> Received for publication Feb. 7, 1924.

<sup>2</sup> The writer is indebted to Dr. H. B. Humphrey, Dr. C. R. Ball, and Dr. H. Hasselbring for their helpful criticisms of the manuscript.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 735.

## RESULTS

## TITRATABLE-ACID CONCENTRATION IN RELATION TO STAGE OF DEVELOPMENT

As might be expected in view of the high buffer content of plant juices, more striking changes with the plant's development were found to occur in the concentration of titratable acid than in the concentration of free hydrogen ions in the juices from these plants. The titration results therefore will be presented first, though the hydrogen-ion determinations are not without interest and will be given in the section following.

Seed of the first series (A) of plants to be grown to maturity was sown on November 1, 1922,<sup>4</sup> in a greenhouse bench containing limed sandy-loam soil of slightly alkaline reaction ( $P_H$  7.2). Acidity determinations were made on sap of each variety at frequent intervals from the time the plants were about 3 inches high until they were in the flowering stage at the age of approximately six months. After that time the measurements had to be discontinued because the plants became so dry that the requisite quantity of juice could not be expressed. The course of the changes in the titratable acidity of all six varieties during this period is shown in figure 1. The age of the plants was always reckoned from the date of sowing. Owing to the diurnal acidity changes reported to occur in many

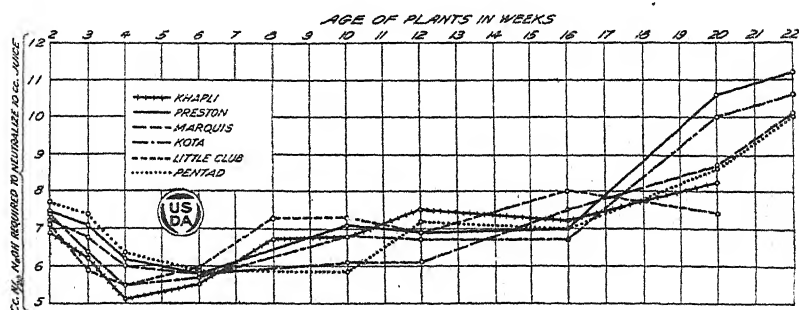


FIG. 1.—The titratable-acid concentration of wheat juice from plants of Series A, from the seedling to the late-flowering stage. (Greenhouse, Nov. 1, 1922, to Apr. 3, 1923.)

plants, only values obtained from plants cut about 1 o'clock in the afternoon were used in order to insure direct comparability of all points on the curves.

The first experiment was repeated with portions of the same seed lots sown three weeks later on another bench of the same greenhouse. The additional precaution was taken of making smaller plots and duplicating the sowing of each variety in order to lessen the chance of error from inequalities in the light, temperature, or water supply along the bench. The plants of this series (B) were somewhat more vigorous than those of Series A because of more favorable temperature and moisture conditions, but their rate of development was practically the same. To eliminate the effects of cutting at different times of the day, only values obtained for plants cut at 9 o'clock in the morning are plotted in figure 2.

<sup>4</sup> Seed for Series A and B was obtained by J. H. Martin from the Agricultural Experiment Stations at Dickinson, N. Dak. (Khapli, C. I. 5378, Preston, C. I. 308r, Pentad, C. I. 3322, Marquis, C. I. 364r), Akron, Colo. (Khapli, C. I. 4013) and Moro, Oreg., (Little Club, C. I. 4066).

The plants of the third series (C) were grown to determine whether the correlation shown by the graphs in figures 1 and 2 to exist between acidity of the juice and stage of development would appear if the plants were in a different environment. Seed of the same varieties was obtained from a different source<sup>5</sup> and sown on February 8 in another

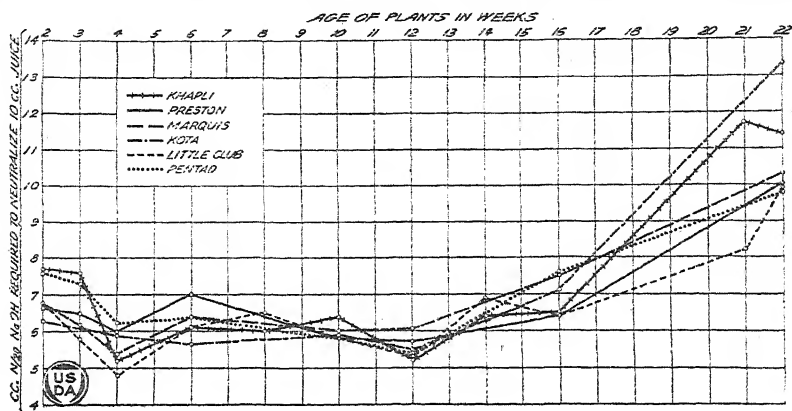


FIG. 2.—The titratable-acid concentration of wheat juice from plants of Series B, from the seedling to the late-flowering stage. (Greenhouse, Nov. 22, 1922, to Apr. 25, 1923.)

greenhouse having unlimed, sandy-loam soil with a reaction of  $P_H$  6.8. The plants developed much more rapidly than those of the two preceding series. They reached the flowering stage in 14 to 16 weeks, whereas the plants of the same varieties in the fall-sown series had required 22 weeks to reach this stage. After 16 weeks, the plants no longer yielded the

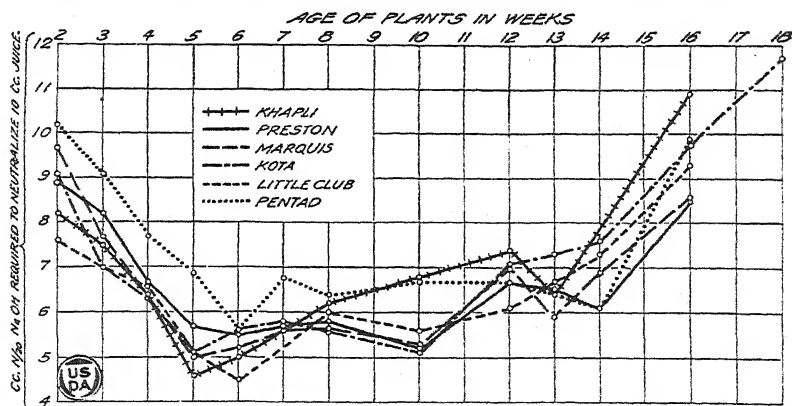


FIG. 3.—The titratable-acid concentration of wheat juice from plants of Series C, from the seedling to the late-flowering stage. (Greenhouse, Feb. 8 to June 14, 1923.)

requisite amount of juice for titration except in the case of Kota where one later measurement was made. Some of the acidity values plotted in figure 3 were obtained from plants cut in the morning and others from plants cut in the afternoon, as indicated in Table I.

<sup>5</sup> Seed of Kota, C. I. 5878, Preston 3081, Pentad C. I. 3322, and Marquis C. I. 3641, for Series C was sent by M. N. Levine from University Farm, St. Paul, Minn., and Khapli C. I. 4013 and Little Club C. I. 4066 by Dr. E. F. Gaines, from Pullman, Wash.

The major trends in the curves of figure 3 are essentially the same as those of figures 1 and 2 and therefore establish the fact that the acidity of the wheat plant undergoes certain regular changes during development from the seedling stage to maturity and that these changes are not environmental effects but the results of inherent physiological characteristics. There is a progressive decrease in acid during the early-seedling stage of the plants of all varieties, with the lowest concentration reached between the ages of 4 and 6 weeks. Then there follows an intermediate period, continuing up to the visible approach of maturity, during which no regular trend is discernible in the curves but only irregular fluctuations. These fluctuations are obviously correlated with variations in the daily environment, as they differ in each series and are often conspicuously parallel for those varieties which were always cut at the same time. During the stage immediately preceding maturation when the culms turn yellow, the lower leaves dry and turn yellow, and head formation begins, the curves turn rather sharply upward, and indicate a steadily increasing acidity. By the time the heads have reached the soft-dough stage, the acid concentration is as great or greater than the maximum found in the seedling stage.

In each series the final upward slopes of the acidity curves were correlated with the drying out of the culms and leaves, the acidity remaining low as long as the plants remained succulent. In other words, the acid concentration during the period of head and flower formation is apparently determined by the loss of water from the tissues. For instance, in Series A and B the heads did not appear in the boot until the plants were partially dry, yet the acidity had begun to rise while the plants were still in the shooting stage. In Series C, on the other hand, in which development was more rapid and the head appeared in the boot while the plants were still vigorous and juicy, the acidity remained low during the period of head formation and did not rise until the flowering stage when the plants began to dry.

Incidentally, Khapli was usually one of the first to head and dry, while the development of Little Club was slower and its maturation more delayed. It follows, therefore, that when this difference in rate of development and drying exists toward the end of the growing period, Khapli will be found to be the more acid in each comparison of values obtained on the same date. In other words, not age alone but also the stage of development determines the acidity of the plants, and this fact must be taken into account in comparisons between varieties which do not mature equally rapidly.

The major trends in the acidity curves are substantially alike in all three series and represent tendencies inherent within the plant, yet environmental factors have left their impress. Higher soil acidity possibly was responsible for the fact that the seedlings of Series C were very much more acid at first than were those of the same age in the other two series. Also, environmental conditions, by determining the rate of development, determined both the duration of the interval elapsing before the beginning of the final ascending portion of each curve and the rate at which the acidity increased during the preripening period.

The foregoing data and discussion relate to healthy plants. When normal growth is prevented by an unfavorable environment, such as that which obtains for a winter wheat grown in the greenhouse during the warm spring months, the acidity curve differs from those given in

figures 1 to 3, inclusive. Figure 4 shows such a curve illustrating the acidity changes occurring in Kanred wheat sown on January 5, 1923, in a greenhouse which was too warm for winter wheat to grow normally. The plants were fairly vigorous in the early-seedling stage, but they were only 4 inches tall at the age of 10 weeks, 8 inches at the age of 13 weeks, and 9 inches at 15 weeks, at which time growth had almost ceased. The leaves remained turgid and green but very narrow, and the stems were very spindling, with almost no tillers. No sign of heading had appeared.

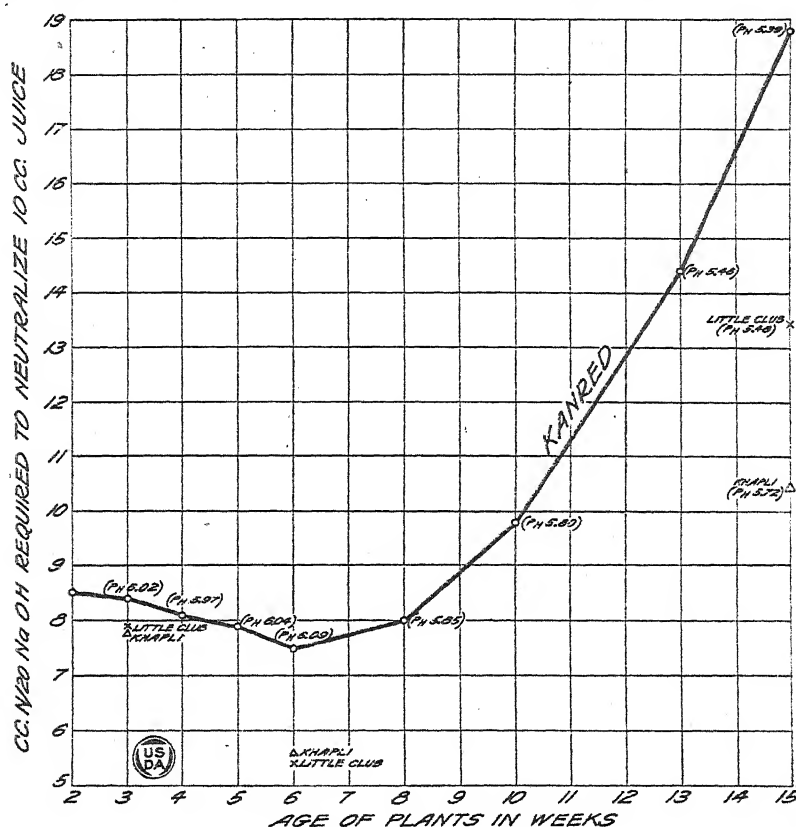


FIG. 4.—The titratable acidity (and pH values in parentheses) of juice of unhealthy Kanred wheat from the seedling stage to cessation of growth, with some corresponding measurements on Khapli and Little Club growing alongside. (Greenhouse, Jan. 5 to Apr. 20, 1923.)

Seed of Little Club and Khapli emmer had been sown at the same time in the same bench. Khapli was flowering at the age of 15 weeks and the acidity measurements, made during the early-seedling, late-seedling, and flowering stages, respectively (fig. 4), were normal values for these stages of growth. Little Club, however, became badly infected with mildew, so that it never headed. By the age of 15 weeks its vigor was visibly affected by the disease, and its unhealthy condition was reflected in the abnormally high acid concentration for that stage of development.

The acidity of Kanred in this environment was very high compared to that of the spring varieties at the corresponding periods. The curve differs from those of the spring wheats in the very slight drop during the

seedling stage and in the steady extreme rise thereafter, coincident with the appearance of symptoms of stunted vegetative growth. The volume of N/20 NaOH required to neutralize a 10 cc. sample of juice increased from 8 to 18.8 cc. in seven weeks. Incidentally, the juice of these plants reached the hydrogen-ion concentration represented by  $P_H$  5.39, which is extraordinarily high for wheat.

#### HYDROGEN-ION CONCENTRATION IN RELATION TO STAGE OF DEVELOPMENT

The  $P_H$  values of the samples, the titratable-acid concentrations of which are given in figures 1 to 3, inclusive, are plotted in figure 5 to show the extent to which the hydrogen-ion concentration of the juice changes with the development of the plant.

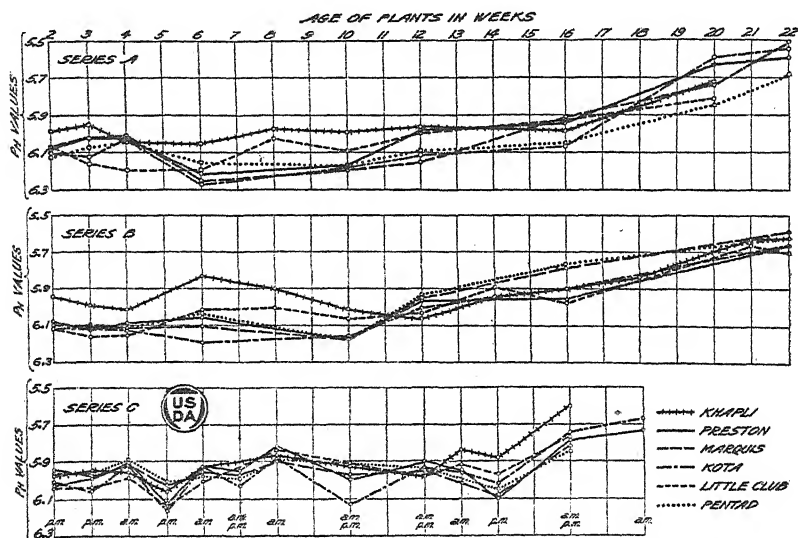


FIG. 5.—The hydrogen-ion concentration of wheat juice from the seedling to the late-flowering stage. The plants of Series C were cut in the forenoon at some periods and in the afternoon at others as indicated.

From these curves it appears that the regular decrease in concentration of titratable acid which has been shown to characterize the juice of wheat seedlings from the age of 2 to about 6 weeks is not accompanied by a correspondingly marked decrease in concentration of hydrogen ions. During the greater part of the plants' development the curves show relatively small and irregular variations which indicate the effects of daily fluctuations in the environment. During the preripening stage, characterized by an increasing titratable-acid concentration, there is a regular upward trend in each hydrogen-ion curve also, indicating that an increasing concentration of hydrogen ions parallels the increase in concentration of titratable acid.

The effects of minor daily fluctuations in environmental factors appear in many of the curves. The existence of these effects is made evident by the conspicuous parallelisms in the courses of certain curves, the corresponding points on which were obtained on the same days throughout. This does not mean that the varieties represented by such curves are more nearly like each other physiologically than like any others of the group, but simply that since the plants of each were always cut at the



same time and handled simultaneously, those external conditions affecting the acidity of one sample also affected that of the other in like degree. It is very interesting to find that the hydrogen-ion concentration of such morphologically unlike varieties as Khapli and Little Club and as Pentad and Marquis should fluctuate so similarly with small changes in environmental factors.

Evidence of the diurnal periodicity in the hydrogen-ion concentration of these plants appears in the curves of Series C in figure 5 and in the corresponding data in Table II from which the curves were plotted. The curves for all the varieties in this series fluctuate up and down together because at some periods all the acidity determinations were made on plants cut in the morning and at others on plants cut in the afternoon, as indicated on the graph. Reference to figure 3 will show that no effect of the difference in the hour at which the plants were cut is discernible in the corresponding titratable-acid values of these same samples. No such fluctuations appear in the hydrogen-ion curves of Series A and B of figure 5 because these were plotted from measurements all of which were obtained from plants cut at 1 p. m. in Series A and at 9 a. m. in Series B.

#### SIGNIFICANCE OF THE DATA FOR THE PROBLEM OF DISEASE RESISTANCE

The variability in the response of all wheat varieties except Khapli and Little Club to attack by the many different specialized races of stem rust now known (7) would seem to preclude *a priori* the responsibility of any one character, such as acidity. Moreover, the data of the preceding sections, giving evidence of extreme and regular variations in acidity during the development of each of the six varieties studied, together with what is known regarding the relative susceptibility of susceptible varieties at different stages of development, afford proof that high or low acid content does not influence the plant's ability to resist invasion by the stem-rust fungus.

A number of investigators have reported observations on the relative susceptibility of the wheat plant throughout its growing period. Farrer (1) says that the liability of wheat to rust attack begins as soon as the plant has flowered and ends when its foliage begins to change color. Freeman and Johnson (2) found wheat most susceptible to *Puccinia graminis* from the time the heads emerged from the boot until they were in full bloom. Melchers and Parker (5) used both heading plants and seedlings for the determination of varietal resistance of wheat to stem rust. Stakman and Piemeisel (8) state that cereals, including wheat, are usually susceptible at any age up to ripening time. Peltier (6) obtained infection of wheat by *P. graminis tritici* in the seedling, stooling, jointing, and heading stages, and his data indicate that the lowest degree of infection occurred in the seedling stage. So it seems obvious that the development of the fungus is not favored by the low acid concentration characterizing the post-seedling stage nor hindered by the relatively high concentration at flowering time.

Gassner (3) also, as the result of an extended experimental study of the relation between stage of development and degree of susceptibility to attack by *Puccinia graminis*, concludes that the susceptibility of wheat and other cereals increases with age, the most susceptible period occurring between the flowering stage and maturity. Equally interesting, in view of the fact that very young wheat seedlings are more acid than older plants, though usually not so acid as those which have passed the flower-

ing stage, is his observation that very young seedlings are sometimes more susceptible than older plants though not so readily infected as those which have passed the flowering stage. Thus it would appear that, if there be any correlation, the degree of infection of a susceptible variety varies directly rather than inversely with the acid concentration. However, these observations may be wholly unrelated and the apparent correlation accidental. At least it appears that the low acidity of the post-seedling period neither favors infection of susceptible varieties nor breaks down the resistance of resistant ones, nor does the high acidity of the older plants make susceptible varieties less liable to attack in the later stages of development.

The significance of the acidity data for the disease-resistance problem may be considered also from the standpoint of varietal comparisons at each stage of the plant's development. Pentad and Kota are resistant to specialized forms to which Preston and Marquis are susceptible. Khapli is resistant to all specialized forms known and Little Club is susceptible to them all (7). Yet at no period in the growth of the plants from the age of two weeks to maturity was either the titratable-acid or the hydrogen-ion concentration of the expressed juice related to the degree of resistance or susceptibility characterizing the variety. Comparisons are facilitated by the tabular arrangement in Tables I and II of the values from which the curves in figures 1, 2, 3, and 5 were plotted.

TABLE I.—*Titratable acidity of wheat at intervals from the seedling to the late-flowering stage, in cubic centimeters of N/20 sodium hydroxid required to neutralize 10 cc. of expressed juice*

SERIES A <sup>a</sup>

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2.....	7.4	7.5	7.7	7.2	6.9	7.1
3.....	6.4	7.1	7.4	6.8	6.2	5.9
4.....	5.5	6.2	6.4	6.0	5.1	5.5
6.....	5.7	5.8	5.9	5.8	5.5	6.0
8.....					6.7	7.3
10.....	6.8	7.1	5.9	6.1	6.8	7.3
12.....	6.7	6.9	7.2	6.1	7.5	6.9
16.....	6.7	7.0	7.0	7.5	7.2	8.0
20.....	10.0	10.6	8.6	8.7	8.2	7.4
22.....	10.6	11.2	10.0	10.1		
24.....			12.0	12.3		
26.....	13.9					

SERIES B

2.....	6.3	6.7	7.6	6.8	7.7	6.8
3.....		6.5	7.3	6.2	7.6	5.8
4.....	5.9	6.0	6.3	5.4	5.2	4.8
6.....	5.7	7.0	6.4	6.4	6.1	6.1
8.....					6.0	6.5
10.....	5.9	5.8	5.8	6.0	6.4	5.8
12.....	5.5	5.7	5.4	6.1	5.2	5.3
14.....					6.4	6.9
16.....	7.1	6.4	7.6	7.5	6.5	6.4
21.....					11.7	8.2
22.....	13.3	10.0	9.8	10.3	11.4	9.9
25.....				11.6		
26.....						12.6

<sup>a</sup> Plants cut at 1 p. m., the others at 9 a. m.

TABLE I.—*Titratable acidity of wheat at intervals from the seedling to the late-flowering stage, in cubic centimeters of N/20 sodium hydroxid required to neutralize 10 cc. of expressed juice—Continued*

## SERIES C

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2.....	<sup>a</sup> 9.1	<sup>a</sup> 8.9	<sup>a</sup> 10.2	<sup>a</sup> 9.7	<sup>a</sup> 8.2	<sup>a</sup> 7.6
3.....	<sup>a</sup> 7.0	<sup>a</sup> 8.2	<sup>a</sup> 9.1	<sup>a</sup> 7.7	<sup>a</sup> 7.5	<sup>a</sup> 7.0
4.....	6.3	6.7	7.7	6.4	6.4	6.6
5.....	<sup>a</sup> 5.1	<sup>a</sup> 5.7	<sup>a</sup> 6.9	<sup>a</sup> 5.0	<sup>a</sup> 4.6	<sup>a</sup> 5.1
6.....	5.6	5.5	5.6	5.2	5.0	4.5
7.....	<sup>a</sup> 5.8	<sup>a</sup> 5.7	6.8	5.6	.....	.....
8.....	5.6	5.8	6.4	5.7	6.2	6.0
10.....	<sup>a</sup> 5.1	<sup>a</sup> 5.2	6.7	5.3	6.3	5.6
12.....	7.1	6.7	6.7	7.0	<sup>a</sup> 7.4	<sup>a</sup> 6.1
13.....	7.3	6.5	.....	5.9	6.4	6.7
14.....	<sup>a</sup> 7.6	<sup>a</sup> 6.1	<sup>a</sup> 6.1	<sup>a</sup> 6.9	.....	<sup>a</sup> 7.3
16.....	9.8	8.5	<sup>a</sup> 9.9	<sup>a</sup> 8.6	10.9	9.3
18.....	11.7	.....	.....	.....	.....	.....

TABLE II.—*P<sub>H</sub> values of the expressed juice of wheat at intervals from the seedling to the late-flowering stage*SERIES A<sup>a</sup>

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2.....	6.10	6.08	6.12	6.07	5.98	6.07
3.....	6.12	6.02	6.07	6.02	5.95	6.16
4.....	6.03	6.02	6.04	6.01	6.04	6.19
6.....	6.27	6.21	6.15	6.25	6.05	6.19
8.....	.....	.....	.....	.....	5.97	6.02
10.....	6.13	6.17	6.17	6.19	5.99	6.09
12.....	6.11	5.98	6.09	6.15	5.96	5.99
16.....	6.06	5.94	6.05	5.91	5.98	5.93
20.....	5.59	5.63	5.84	5.73	5.72	5.81
22.....	5.54	5.59	5.68	5.50	.....	.....
24.....	.....	.....	5.57	5.53	.....	.....
26.....	5.58	.....	.....	.....	.....	.....

## SERIES B

2.....	6.11	6.07	6.11	6.09	5.94	6.11
3.....	.....	6.12	6.09	6.10	5.99	6.16
4.....	6.12	6.08	6.11	6.11	6.01	6.15
6.....	6.19	6.05	6.03	6.10	5.83	6.01
8.....	.....	.....	.....	.....	5.90	6.00
10.....	6.16	6.17	6.17	6.17	6.01	6.06
12.....	6.00	5.96	5.93	5.95	6.06	6.03
14.....	.....	.....	.....	.....	5.94	5.89
16.....	5.90	5.95	5.76	5.78	5.90	5.97
18.....	.....	.....	.....	.....	5.83	.....
21.....	.....	.....	.....	5.62	5.62	5.66
22.....	5.66	5.66	5.62	5.58	5.62	5.70
26.....	.....	.....	.....	.....	.....	5.69

<sup>a</sup> Plants cut at 1 p. m., the others at 9 a. m.

TABLE II.—*P<sub>H</sub>* values of the expressed juice of wheat at intervals from the seedling to the late-flowering stage—Continued

## SERIES C

Age in weeks.	Kota.	Preston.	Pentad.	Marquis.	Khapli.	Little Club.
2.....	<i>a</i> 6.01	<i>a</i> 6.03	<i>a</i> 5.96	<i>a</i> 5.94	<i>a</i> 5.98	<i>a</i> 6.04
3.....	<i>a</i> 6.06	<i>a</i> 5.99	<i>a</i> 5.96	<i>a</i> 5.98	<i>a</i> 5.95	<i>a</i> 6.04
4.....	5.92	5.91	5.89	5.92	5.96	5.97
5.....	<i>a</i> 6.17	<i>a</i> 6.12	<i>a</i> 6.01	<i>a</i> 6.03	<i>a</i> 6.06	<i>a</i> 6.14
6.....	5.93	5.93	5.98	5.96	5.93	6.01
7.....	<i>a</i> 6.03	<i>a</i> 5.95	5.99	5.97	.....	.....
8.....	5.89	5.82	5.89	5.90	5.87	5.84
10.....	<i>a</i> 6.13	<i>a</i> 6.00	5.91	5.97	5.93	5.91
12.....	5.94	5.90	5.94	5.95	<i>a</i> 5.98	<i>a</i> 5.98
13.....	5.96	5.95	.....	6.02	5.84	5.92
14.....	<i>a</i> 6.02	<i>a</i> 6.10	<i>a</i> 6.05	<i>a</i> 6.08	<i>a</i> 5.88	<i>a</i> 5.97
16.....	5.74	5.78	<i>a</i> 5.85	<i>a</i> 5.82	5.60	5.77
18.....	5.67	5.73	.....	.....	.....	.....

*a* Plants cut at 1 p. m., the others at 9 a. m.

With the exception of the relatively high titratable-acid concentration of the young seedlings of Pentad in Series C, and the relatively high hydrogen-ion concentration of Khapli, up to the age of about 10 weeks, in Series A and B, the values in Tables I and II give no evidence of significant varietal differences, but rather of surprisingly close agreements for varieties so unlike, both as to morphological type and susceptibility to disease. Since these few varietal differences do not occur in all three series they probably represent differences in the reaction of the variety to the particular environment and are without significance for the disease-resistance problem. The data as a whole confirm the earlier conclusion (4) that the hydrogen-ion concentration of wheat juice bears no relation to varietal resistance to stem rust, and permit the same conclusion with respect to titratable-acid concentration.

## CONCLUSIONS

The titratable acidity of the juice of the wheat plant undergoes a regular sequence of changes during development from the seedling stage to maturity. There is a progressive decrease, sometimes to half the initial concentration, between the ages of two and about six weeks. This period is followed by a period of relatively low acidity, with minor fluctuations, extending up to the visible approach of maturity, at which time the acid concentration rises as the plants ripen and dry. The final value may be twice the highest seedling concentration and almost three times that of the least acid stage.

The hydrogen-ion concentration of the juice of the wheat plant does not decrease appreciably between the ages of two and six weeks. It is greatly increased during the preripening period and reaches a relatively high value at the flowering stage and later.

This increasing acid concentration during the final stages of growth is correlated with the rate of drying rather than with head formation or kernel development.

Both the titratable-acid and hydrogen-ion concentrations are influenced by environmental conditions which determine the rate of growth and

which bring about daily fluctuations in acidity, but these variations are insignificant in comparison with the changes correlated with the changing stage of development of the plant, so that the major trends in the acidity curves persist clearly under all conditions.

Stunted, slow-growing plants, such as those of Kanred in a warm greenhouse, are characterized by an extremely high titratable-acid and hydrogen-ion concentration and may not have the intermediate period of low acidity. Also, infection by mildew, when severe enough to visibly affect the vigor of the plant, results in an abnormally high acidity.

The validity of varietal comparisons based on acidity measurements may be open to question unless the plants are at the same stage of development as well as of the same age, equally vigorous, and subjected throughout their development to the same environmental conditions.

Varietal resistance to stem rust is not related at any stage of development to titratable-acid or hydrogen-ion concentration.

High acidity of the juice does not hinder attacks of the stem-rust organism, for investigators have found that the wheat plant is as susceptible during the heading and flowering stages as it is during the earlier periods of low acidity. Conversely, low acidity does not predispose to the disease, since the plant is no more liable to infection during the period of lowest acidity than it is during the earlier and later stages. Moreover, resistant varieties pass through the period of low acid concentration at the same stage as do the susceptible ones and no breakdown in their resistance at this time has been reported.

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## CYTOLOGICAL STUDIES OF DIPLOID AND POLYPLOID FORMS IN RASPBERRIES<sup>1</sup>

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This investigation of the raspberries of the subgenus *Idaeobatus*<sup>2</sup> is a continuation of cytological studies of the pollen-mother-cell development of *Rubus* begun by the senior author in 1921. In the first investigation, a report of which is now in press, the senior author found that diploid forms, that is, forms with a reduced number of seven bivalent chromosomes, were the exception in the subgenus *Eubatus*.

The present article presents the result of a careful study of the pollen-mother-cell development of species and varieties used in raspberry breeding by the Office of Horticultural Investigations of the United States Department of Agriculture, in which it is shown that most raspberries have seven bivalent chromosomes.

The study was undertaken to determine the chromosome number of the various species of raspberries as an aid to breeding and especially to find some explanation for the peculiar behavior of hybrids of La France and Ranere (*St. Regis*) varieties. From 100 or more seedlings of this particular cross only one weak plant gave well-developed fruit. A large percentage (32) bore sterile flowers and the remainder showed a range in fertility from those having an occasional flower in which a drupelet set to those with several drupelets to each flower. La France has been considered a variety of *Rubus idaeus*, the European raspberry, and Ranere, a variety of *R. strigosus*, the American red raspberry. These species are more closely related than many others which, when hybridized, give all, or nearly all, fertile progeny. For example, hybrids of Gregg (*R. occidentalis*) × Ranere (*R. strigosus*) in the same environment as La France × Ranere hybrids are entirely fertile. Certain other forms, when crossed, also gave an unexpectedly large percentage of infertile seedlings. Such results are difficult to interpret and it was hoped that knowledge of chromosome behavior might help to explain them.

The subgenus *Eubatus* (blackberries) in which the haploid number is seven has many chromosome groups, that is, tetraploid, pentaploid, hexaploid, and octaploid (12). The closely related genus *Rosa* also has the same basic chromosome number seven and has similar chromosome groups (19) (3) (14). Hybrids between polyploidal forms of *Rosa* are reported to behave in a manner quite different from the simple Mendelian expectation. It was considered possible, therefore, that there were similar polyploidal groups among the raspberries which would explain the results observed. To make the work as systematic, compre-

<sup>1</sup> Received for publication Feb. 9, 1924.

<sup>2</sup> The classification used in this paper is, for the most part, that of Focke given in "Species Ruborum" (8).<sup>3</sup>

<sup>3</sup> Reference is made by number italic to "Literature cited," p. 747-748

hensive and helpful as possible, representatives of as many types of raspberries as could be procured were selected with special reference to the economic importance of the groups, their botanical relationship, and their possible value in breeding.

### MATERIAL AND METHODS

The plants from which buds were taken were grown at the Bell Horticultural Field Station, Glendale, Md., and at Arlington Experiment Farm, Rosslyn, Va.

Material was collected during the last two weeks of May, in June, and again in September and October (1923). Buds were killed in weak chromo-acetic solution, and in Bouin's killing mixture, both of these being quite satisfactory.

All material was imbedded in nitrocellulose, this medium having been found very satisfactory for this type of investigation. The buds were sectioned about 15 micra thick so as to have uncut nuclei for study.

Heidenhain's haematoxylin was used for staining chromosomes. The mother cells were studied by the use of 2 mm. and 1.6 mm. Zeiss apochromatic lenses and No. 12 and 18 compensation oculars.

Pollen counts were made from anthers that had not dehisced. Plate 1, A, shows a bud in the stage of development usually chosen. The anthers were crushed in water on a glass slide and all plump grains counted as good. As a rule, the count was made from 100 grains only and the percentage of good pollen is, therefore, only approximate. Germination tests and special technique are necessary for a final test.

### HISTORICAL AND CYTOLOGICAL DESCRIPTION

For convenience, the classification of Focke has been followed, for the most part, and the results have been presented in sections and series as given in "*Species Ruborum*" (8). The few polyploid forms, however, have been segregated and discussed after the review of the diploid forms.

Brief historical descriptions of many raspberry species and garden varieties are first given. The inconsistency existing in the nomenclature of horticultural raspberry and blackberry forms makes it necessary to include the available history of each species and variety used in this study, since in this way the identity of the material will be established more definitely. These descriptions, which we regret are incomplete, are followed by the results of morphological and cytological studies of pollen and pollen formation.

Table I presents a summary of the pollen counts and the chromosome number and behavior during the pollen tetrad formation of the species and varieties studied. Plates 2 and 3 show drawings of heterotypic prophase selected from type species, varieties, and hybrid forms.



TABLE I.—Chromosome number and pollen condition of raspberries

	Percentage of good pollen.	Number of haploid chromosomes.	Behavior of chromosomes at meiosis.	Plate No.
Subgenus <i>Idaeobatus</i> :				
Section <i>Rosaeifolii</i> —				
1. <i>Rubus idaeobatus</i> .....	97	7	Regular.....	
2. <i>R. thunbergii</i> .....	86			
Section <i>Pungentes</i> —				
3. <i>R. lasiostylus</i> .....		7	do.....	2 H
4. <i>R. lasiostylus</i> <i>dizygos</i> × <i>R. idaeus</i> horticultural variety Superlative.....	40	7	do.....	2 K
Section <i>Idaenanthi</i> —				
Series <i>Nivei</i> —				
5. <i>R. coreanus</i> .....		7	do.....	2 I
6. <i>R. coreanus</i> × <i>R. strigosus</i> horticultural variety Newman.....		7	do.....	2 L
7. <i>R. phoenicolasius</i> .....		7	do.....	2 O
Series <i>Thrysidai</i> —				
8. <i>R. adenophorus</i> .....		7	do.....	2 P
9. <i>R. innominatus</i> .....		7	do.....	2 J
10. <i>R. innominatus</i> × <i>R. idaeus</i> horticultural variety Superlative.....		7	do.....	2 M
11. <i>R. innominatus</i> × ( <i>R. idaeus</i> × <i>R. strigosus</i> ) horticultural variety Cuthbert <sup>a</sup> .....	33	7	do.....	2 N
Series <i>occidentalis</i> —				
12. <i>R. occidentalis</i> —				
Horticultural variety Cumberland.....		7	do.....	2 C
Horticultural variety Farmer.....		7	do.....	
Horticultural variety Gregg.....	98	7	do.....	
13. <i>R. occidentalis</i> × <i>R. idaeus</i> —				
Horticultural variety Gregg × Wisbeck.....		7	do.....	2 E
Horticultural variety Royal.....		7	do.....	
14. ( <i>R. occidentalis</i> × <i>R. idaeus</i> ) × <i>R. strigosus</i> —				
Horticultural variety Cardinal.....	75	7	do.....	
Horticultural variety Royal × Newman.....		7	do.....	2 G
15. <i>R. occidentalis</i> × <i>R. strigosus</i> —				
Horticultural variety Gregg × King <sup>b</sup> .....	93	7	do.....	2 F
Series <i>Eu-Idaea</i> —				
16. <i>R. mesogaeus</i> .....	89	7	do.....	2 Q
17. <i>R. idaeus</i> —				
Horticultural variety Lloyd George.....	99	7	do.....	2 B
Horticultural variety Magnum Bonum.....				

<sup>a</sup> The classification of raspberry varieties used is that of Darrow; see literature cited (6).<sup>b</sup> The pollen count was made from one of the most fertile forms of this cross.

TABLE I.—Chromosome number and pollen condition of raspberries—Continued

	Percentage of good pollen.	Number of haploid chromosomes.	Behavior of chromosomes at meiosis.	Plate No.
Subgenus <i>Idaeobatus</i> —Continued.				
Section <i>Idaenanthi</i> —Continued.				
Series <i>Eu-Idaea</i> —Continued.				
18. <i>R. idaeus</i> (?)—				
Horticultural variety				
La France.....	56	14	Irregular...	3 B
Horticultural variety				
Merveille Rouge....	24	14	...do.....	3 E
Horticultural variety				
Merveille de Quatre				
Saisons Rouge.....	28	14	...do.....	3 F
Horticultural variety				
Surpasse Merveille a				
blanc.....	10	14	...do.....	3 G-I
Horticultural variety				
Souvenir de Desire				
Bruneau.....	36			
Horticultural variety				
All Summer.....	97	21/2	...do.....	
Horticultural variety				
White Queen.....	96	21/2	...do.....	
19. <i>R. idaeus</i> × <i>R. strigosus</i> —				
Horticultural variety				
Cuthbert.....	70	7	Regular...	2 D
Horticultural variety				
Marlboro.....	50			
20. <i>R. idaeus</i> × <i>R. strigosus</i>				
(?)—				
Horticultural variety				
La France × Ranere..	Variable.	21/2	Irregular...	3 C
Horticultural variety				
Erskine.....	50	21/2	...do.....	3 D
21. <i>R. strigosus</i> —				
Wild Plant.....	100	7	Regular...	2 A
Horticultural variety				
Eaton.....		7	...do.....	
Horticultural variety				
King.....		7	...do.....	
Horticultural variety				
Newman.....		7	...do.....	
Horticultural variety				
Ranere.....	100	7	...do.....	3 A

## DIPLOID RASPBERRIES

## SECTION PUNGENTES × IDAENTHI

*R. lasiostylus* dizygous × *R. idaeus* horticultural variety Superlative. This cross was made by the junior author at the Bell Horticultural Field Station and buds were taken from a selected individual.

## SECTION IDAENTHI

*R. coreanus* × *R. strigosus* horticultural variety Newman. This cross was made by the junior author at the Bell Horticultural Field Station and the buds taken from a selected bush. The *R. coreanus* parent of this hybrid was grown from seed secured from Kew, England, but itself seems to be a hybrid, *R. coreanus* × *R. biflorus*.

*R. innominatus* S. Moore. The plants of this species were propagated from plants grown by the late Dr. Walter Van Fleet, presumably from seed sent by E. H. Wilson from China.

*R. adenophorus* Rolfe. The plants of this species were obtained from the Office of Foreign Seed and Plant Introduction, United States Department of Agriculture, under No. 52939.

*R. innominatus* × *R. idaeus* horticultural variety Superlative. The plant from which buds were taken was one of several of this cross made by the junior author at the Bell Horticultural Field Station.

*R. innominatus* × (*R. idaeus* × *R. strigosus*) horticultural variety Cuthbert. Horticultural variety Van Fleet. This hybrid was made by the late Doctor Van Fleet at Chico, Calif., and the plants grown from seed at the Bell station.

*R. occidentalis* Horticultural variety Cumberland. This was originated by David Miller of Camp Hill, Pa., and introduced in 1898.

Horticultural variety Farmer (Plum Farmer). This was found in a shipment of another variety received by L. J. Farmer, Pulaski, N. Y., and introduced by him in 1895. Farmer, as an early sort, and Cumberland as a late variety are the leading black raspberries grown in the United States.

*R. occidentalis* × *R. idaeus*. Horticultural variety Gregg × Wisbeck. The buds were collected from one of several seedlings of this cross made by the junior author at the Bell station.

Horticultural variety Royal. This variety was originated in Indiana by L. H. Gerton, and was introduced by L. J. Farmer, Pulaski, N. Y., in 1900.

(*R. occidentalis* × *R. idaeus*) × *strigosus*. Horticultural variety Cardinal. This variety originated on the place of A. H. Griesa, Lawrence, Kans., in 1888, apparently from seed of the Shaffer. Because of its glandular hairy inflorescence it is considered a cross of the Shaffer with a variety of *R. strigosus* (4).

Horticultural variety Royal × Newman. The material for study was taken from a plant growing at the Bell station which was one among the many crosses of this parentage made by the junior author.

*R. occidentalis* × *R. strigosus*. Horticultural variety Gregg × King. This material for study was taken from one plant, which was selected for its good fruit, from a large number of plants of this cross made by the junior author, at the Bell station. Most seedlings of this cross were partly sterile, setting but few drupelets to each flower. This seedling was selected as the best and its pollen count showed a notably high percentage of good pollen grains. The seed parent was a variety of black raspberry resembling Gregg but concerning the identity of which there was some question. The abundant glandular inflorescence clearly distinguishes these crosses from the Royal and the Gregg × Wisbeck.

*R. idaeus*. Horticultural variety Lloyd George. This variety was found wild and introduced by J. H. Kettle, Corfe Mullen, Winborne, Dorset, England, in 1920.

Horticultural variety Magnum Bonum. This old variety of Europe was introduced into America about 1840. It differs markedly from the other European raspberries which we have studied. The turions are densely covered with glandular bristles and it resembles mountain and maritime forms.

*R. idaeus* × *R. strigosus*. Horticultural variety Cuthbert. This variety originated as a chance seedling in the garden of Thomas Cuthbert at Riverdale, N. Y., about 1865 growing near plants of the Hudson River Antwerp, a variety of *R. idaeus*. It is, therefore, considered a hybrid of *R. idaeus* × *R. strigosus*.

Horticultural variety Marlboro (*Abundance*, *Laxton's Abundance*, *Perfection of England*). The material used was gathered from plants received as Laxton's *Abundance* from Laxton Bros., England, a variety which was determined to be identical with Marlboro by Grubb (9). This is confirmed by our examination. Marlboro was originated by A. J. Caywood, Marlboro, N. Y., as a cross of Highland Hardy, and a seedling from English Globe and the Hudson River Antwerp (5), and introduced in 1884. Highland Hardy is supposed to be a variety of *R. strigosus*. November *Abundance* is probably a distinct sort, having originated in England.

*R. strigosus* Mchx. Wild plants from Hebron, N. Y. In September, 1920, selections were made of bushes fruiting freely in the fall on turion tips. These were transplanted to the collection of the Bell Horticultural Field Station.

Horticultural variety Eaton. Found as a chance seedling at Cambridge City, Ind., in 1885. Its glandular inflorescence refers it to *R. strigosus*.

Horticultural variety King. The history of this variety is not entirely clear. It is widely grown in the upper Mississippi Valley States. The buds used were from plants of this variety secured from Michigan. It is supposed to be the Thompson's King sent out by the Cleveland Nursery Co. of Rio Vista, Va., in 1892, which variety was grown from seed of Thompson by T. Thompson of Richmond. Beach, in 1895, however, states of this variety, "Canes vigorous, show evidence of *Idaeus* parentage" (2, p. 204). King, as grown to-day, does not show evidence of *R. idaeus* parentage, except perhaps in that a large percentage of its progeny are more or less infertile, but it has the glandular inflorescence, the thin leaves, and light-red fruit of *R. strigosus* and is very hardy. Over 100 hybrids with a black raspberry and many hundred other hybrids and crosses with it made by the junior author fail to show characteristics of *R. idaeus* but do show characteristics of *R. strigosus* and it is classified accordingly.

Horticultural variety Newman. This variety was grown from seed of Eaton, by C. P. Newman, La Salle, Quebec, and was introduced by the Provincial Government of Quebec, Canada, in 1921. Its hardiness and light red fruit refer it to this species. Newman states that the pollen parent may have been King.

Horticultural variety Ranere (*St. Regis*). The Ranere was found wild near Hammonton, N. J., by A. Ranere and was extensively grown by him and others several years before 1910 when it was introduced under the name "St. Regis." It is densely glandular, hairy, very hardy, has thin leaves and light red fruit, and is referred to *R. strigosus* (6).

Table I shows that all raspberries examined cytologically can be separated into two classes, those with seven haploid chromosomes, and those with more than seven.

Such a division also separates all species into two groups based on the distribution of the chromosomes during the reduction divisions of the pollen mother cell. The diploid species, that is, those showing seven bivalent or haploid chromosomes just previous to the first reduction division, have the somatic or univalent chromosomes pairing very promptly in the prophase of the first reduction division, and these bivalent chromosomes divide regularly and promptly in both the first and second divisions. Such a division gives a quantitatively equal distribution of all chromatin material to the four daughter nuclei, and consequently this type of division has been termed regular. However, a study of the reduction phases showed, in rare cases, a little tardiness in pairing of univalent chromosomes, or occasionally a single chromosome lagging behind its associates in reaching the pole after either the first or the second reduction division but these irregularities were so rare that they may be disregarded.

#### POLYPLOID RASPBERRIES

The following section is a discussion of a smaller group of horticultural varieties of raspberries in which the chromosome number is larger than is the rule in diploid forms and in which chromosomes show characteristic irregularities in their distribution during meiosis.

*R. idaeus* (?). Horticultural variety La France. This variety was secured from the introducer, John Scheepers Co., New York City. The original stock was introduced from France probably between 1890 and 1900 and grown at Stamford, Conn., from which place it was distributed. It resembles Merveille de Quatre Saisons Rouge very closely and may be identical with it.

Horticultural variety Merveille Rouge. This old French variety was raised and introduced by Simon Louis Frères of Metz, France. Our stock which was secured from Laxton Bros., Bedford, England, is very similar to Merveille de Quatre Saisons Rouge.

Horticultural variety *Merveille de Quatre Saisons Rouge*. This is probably the same as *Perpetual de Billiard*. It was raised and introduced in 1849 by M. Billiard, nurseryman of Fontenay, near Paris. Stock of this was obtained from Orleans, France. It is one of the old varieties and is reported as identical with *October Red* (20) and the *Old Double-Bearing* (18). It is also reported as a supposed seedling of *Fastolff* (16).

Horticultural variety *Surpasse Merveille a Blanc*. Stock under this name was secured from Orleans, France, but it bears red fruit and resembles *Merveille de Quatre Saisons Rouge* very closely and its probable identity with this variety is indicated further by this cytological study.

Horticultural variety *All Summer*. Plants of this variety were obtained from Orleans, France.

Horticultural variety *White Queen*. *White Queen* was introduced in 1920 by Wm. M. Hunt & Co., of New York City, who state that it may be of French parentage. It was found on the place of Jonathan Thorne at Black Rock, Conn., and is supposed to be a chance seedling. It fruits freely in the autumn on young canes.

*La France* was the first raspberry studied in which more than the 7 haploid chromosomes were found and, because of this, all phases of meiosis have been studied critically. Plate 3, B, pictures the 14 bivalent chromosomes. They appear crowded in the drawing, but are on the periphery of the nucleus and quite separate in the actual observations. Not only is there an increase in chromosome number above that of diploid forms, but there is also a change in the general appearance of the anthers. Clear figures, plump mother cells and prompt pairing of the chromosomes at diakinesis are absent and one has to study much material and stain carefully in order to overcome the unfavorable conditions found in this polyploid form during meiosis.

It seems unnecessary to discuss in detail the reduction divisions of this variety but one characteristic should be mentioned, that is, the irregular distribution of chromosomes, some of which lag on the spindle and are extruded from the daughter nuclei. Such behavior is believed to indicate hybrid origin, and results in the formation of pollen grains with varying chromosome numbers and varying in viability.

*La France*, though not hardy, endures the winters better than many European raspberry varieties. It has dark green foliage which is more resistant to leaf spot than spring fruiting European sorts. It also bears fruit quite freely on the turion tips in the autumn. Five varieties with characteristics similar to *La France* were selected and five additional polyploidous forms were found. The cytological study of these forms had associated with it the difficulties referred to in the foregoing variety. The mother cells frequently were thin and vacuolated and collapsed before they reached the tetrad stage. After much search favorable figures of many stages were found.

The 14 haploid chromosomes of *Merveille Rouge* and *Merveille de Quatre Saisons Rouge* are represented in Plate 3, E and F. These typical prophase resemble that pictured for *La France* and the later phases have associated with them the same interesting irregularities. Consequently, one would expect to find a large amount of pollen sterility, an expectation borne out by the actual observations, both varieties having only 25 per cent viable pollen.

*Surpasse Merveille a Blanc* has 14 bivalent chromosomes. The early prophase which showed the univalents grouped in pairs were very favorable for study. Plate 3, G was drawn from a figure that had only three bivalent chromosomes, the remaining 22 univalents are seen grouped in pairs. A later prophase showing only bivalent chromosomes is represented in Plate 3, H. Plate 3, I, is a drawing of a very late heterotypic

prophase or early metaphase. Seven chromosomes are at the equatorial plate, some even having divided, while the remaining 7 are lagging in their approach to the plate. It is these laggards that cause observed irregularities in this and the homotypic division. They are again tardy in their movement from the nuclear plate and may not be included in the daughter nuclei and so the resulting pollen grains do not receive either a qualitatively or quantitatively equal amount of chromatin material. This type of reduction is styled as irregular.

The chromosome number of varieties White Queen and All Summer seems to be  $\frac{21}{2}$ . During meiosis they show many of the irregularities peculiar to triploid hybrids, and, consequently, they are grouped with the foregoing polyploidous raspberries. The pollen of these two sorts, however, seems remarkably good when their chromosome condition is considered.

*R. strigosus*  $\times$  *R. idaeus*, (?). Horticultural variety Erskine. This originated at Lee, Mass., with E. J. Norman, being found in 1895 among Marlboro plants set in 1890. Two other varieties, the Cuthbert and Golden Queen, were being grown by Norman at that time. As the plant was small, he supposed it to be a seedling of the Marlboro. The Marlboro plants were obtained from Ellwanger & Barry of Rochester, N. Y., who also were growing Fontenay and other European raspberries. Fontenay is an autumn-fruited sort and probably belongs to this polyploid group. There is a bare possibility that Erskine may have come from that source. If the assumption that Erskine originated from the Marlboro is correct then it is the only polyploid variety of American origin derived from American varieties yet found.

Horticultural variety La France  $\times$  Ranere. These hybrids were made by the junior author at the Bell Horticultural Field Station and two of the seedlings, one entirely sterile, the other nearly so, were selected for this study.

Erskine was the second polyploid raspberry discovered and difficulty was experienced in finding good mitotic figures to study the various phases of this form. This supposed hybrid is triploid, and the reduced chromosome number is represented in Plate 3, D. There are generally 10 chromosomes at diakinesis. In the earlier study of the Eubatus subgenus by the senior author, triploid forms were found to be very abundant, and this hybrid behaves in a manner very similar to that described for triploid blackberries. Seven of the bivalent chromosomes behave in a regular manner during the reduction phases, but the remaining chromosomes are slow about fusing in the heterotypic prophase. These laggards are distributed, during meiosis, in an irregular manner to the four daughter nuclei or are frequently extruded into the cytoplasm where they degenerate or become the nuclei for dwarf pollen grains.

The hybrid, La France  $\times$  Ranere, is triploid, which would be the natural result if the parents contributed 14 and 7 chromosomes, respectively. Plate 3, A, B, and C, represents prophases of the parents and the hybrid. This opportunity to study an  $F_1$  raspberry hybrid has shown that it behaves in a manner very similar to that described by Rosenberg (15) for the well-known Drosera hybrid. This artificially produced raspberry hybrid has all the irregularities of chromosome distribution noticed in triploid blackberries, and furnishes additional evidence that many of our blackberries are very recent hybrids.

In collecting material of this La France  $\times$  Ranere cross, buds were gathered from two seedlings, No. 1 and No. 2. All anthers of No. 1, as pictured in Plate 1, C, were so sterile that it was impossible to find normal reduction phases. Such extreme sterility recalls the sterility of *Prunus cerasus*  $\times$  *P. avium* as reported by Dutrochet (7) who writes, "The 'stamina'

formed a compact mass in which no pollen was formed." Form No. 2 was more fertile, as the anthers pictured in Plate 1, B, show. From this plant there was procured sufficient material for a study of the reduction division. Since this hybrid is triploid, it corresponds to the intergroup crosses of wheat (*Triticum*) in which Sax (17) found the greatest sterility. It indicates that the sex cell on La France side had 14 chromosomes. The behavior in egg formation, therefore, differs from that studied by Täckholm for the *Canina* group of roses. He found that the viable egg of a tetraploid species had 21 chromosomes and the viable pollen usually had 7 chromosomes.

It seems certain that this list of polyploid raspberries is not complete. The varieties Buckeye, Hailsham, and Souvenir de Desire Bruneau show external characteristics which place them in this group, that is, as grown in the United States, they are more hardy than the diploid varieties of *R. idaeus*, have heavy dark green foliage, resist leaf spot, and bear fruit on young canes in autumn.

## DISCUSSION

### POLLEN STERILITY IN RASPBERRIES

Table I shows a dozen diploid forms in which the per cent of good pollen has been determined. Five of these diploid varieties are hybrids, and the large amount of sterile pollen existing in them, in contrast to the small amount of sterile pollen that generally exists in stable species, supports the view that hybridizing may cause noticeable sterility in the offspring (10).

This table also shows a second group of sterile raspberries, including all but two of the polyploidous forms. The high percentage of good pollen found in the two exceptions was unexpected, and since a study of these varieties is not complete, no explanation will be attempted. The remaining six forms are very sterile and characterized by an unequal distribution of the chromosomes during the pollen tetrad formation. This extreme sterility may be attributed to unbalanced chromosome conditions existing in the nucleus of the pollen grain, which in turn is recognized as a character of hybrids between incompatible species. The inference, therefore, seems to be that raspberries showing pollen sterility are of hybrid origin and that polyploidous raspberries are the result of crosses between plants belonging to different chromosome groups.

### CHROMOSOME MULTIPLICATION DUE TO HYBRIDIZATION

Polyploidy in raspberries seems to have originated in both Europe and America. Our knowledge is too limited to allow the acceptance of one explanation to the exclusion of all others as to how it originated, but the present data support the theory that polyploidism in raspberries has originated through hybridization.

In England *Rubus caesius*, a tetraploid trailing blackberry, blossoms at approximately the same season as the raspberries, while the common blackberries blossom several weeks later than the usual raspberry flowering season. A species with dark thick leaves, similar to *R. caesius*, seems essential to explain the characters present in polyploid raspberries. One English autumn-bearing raspberry was reported as a hybrid, *R. caesius*  $\times$  *R. idaeus* (11). The junior author has raised many *R. idaeus*  $\times$

*R. caesius* crosses from seed and obtained a great variety of forms, but unfortunately these  $F_1$  plants had been destroyed previous to this study and no cytological material is available.

An  $F_2$  hybrid, originated by Professor Ness (13) of the Texas Experiment Station, is a cross between the Cardinal raspberry, a diploid form, and *R. rubrisetus*, a southern blackberry. A cytological study of this hybrid seems to provide evidence to support the theory of the origin of polyploidism in raspberries through hybridization and selection.

Three figures drawn from early reduction phases of this  $F_2$  hybrid show the cytological conditions in this hybrid. Plate 3, J and K, shows the 14 chromosomes of this tetraploid form, while Plate 3, L, pictures a slight irregularity in chromosome distribution. It was exceptional to find irregularities during meiosis, but the history of this hybrid shows that it has been selected through several generations and now behaves like a stable species.

This  $F_2$  hybrid demonstrates that fertile raspberry-blackberry hybrids are possible and it also shows that a tetraploid *Rubus* has been experimentally produced with the characteristics of a stable species.

After studying many known raspberry  $\times$  raspberry hybrids without finding a single case of chromosome multiplication it seems worth while to consider the possibility that all the polyploidous forms described in this article are the result of raspberry  $\times$  blackberry hybrids that have been selected artificially or naturally and now have found a place in our commercial varieties of raspberries.

#### SIGNIFICANCE OF DIPLOID, TRIPLOID, AND TETRAPLOID RASPBERRIES

We have shown that all the species or varieties of raspberries examined have the basic chromosome number seven. Furthermore, hybrids even between species which are quite different in their external characters also have the same basic number seven. A few varieties referred to *R. idaeus*, however, are triploid or tetraploid and the presence of these few variant forms, in a group otherwise uniform in this respect, is important.

There are genera in which chromosome multiplication has been reported, and in which new hybrid species and varieties are appearing continually. The blackberries, roses, and hawthorns are well known examples where multiplication of species and varieties is associated with chromosome multiplication. In Europe several thousand *Eubatus Rubi* have been collected and described as species while the recent American publication on "Standardized Plant Names" (1), devotes 50 pages to listing varieties of roses alone. The presence of a few polyploid raspberries, of unquestionably recent origin, may be the beginning of a similar multiplication in raspberry forms. All polyploidous raspberries belong to the best autumn-fruited European group, and, therefore, an effort should be made to combine by hybridization their good qualities with those of our hardy American varieties.

La France  $\times$  Ranere hybrids, obtained at the Bell station by the junior author demonstrate the variable results that may be expected from inter-group hybrids. The raspberry breeder who attempts to introduce the characters present in polyploid raspberries by hybridization will find his efforts seriously limited by the incompatibility frequently existing between hybrids involving different chromosome groups.

The recent study of tetraploid *Datura* hybrids (4) is a carefully planned scientific research into the genetic behavior of a recently discovered



group of polyploid forms. This pioneer investigation shows that the chances of obtaining new combinations have increased with an increase in chromosome number and it has been found by studying large populations from such hybrids that the offspring follow certain genetic laws of segregation. It is only by means of such experimental hybridization that the plant breeder, who is working with a genus such as *Rubus*, may help to deduce laws of inheritance that will aid him to combine desirable qualities from the varied material at hand and segregate them in the later generations.

#### SUMMARY

Diploid species and hybrids are the rule in the *Idaeobatis* subgenus of *Rubus*.

Triploid and tetraploid forms are few in number but are a significant and characteristic group of raspberries.

This study suggests that polyploid raspberries are *Idaeobatis* × *Eubatus* hybrids.

Polyploidism, if once established in a group, is likely to increase; consequently the breeder attempting interchromosome group crossing will find his difficulties increased, the interpretation of results more difficult, but the chance of obtaining new combinations of characters multiplied.

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PLATE 1<sup>a</sup>

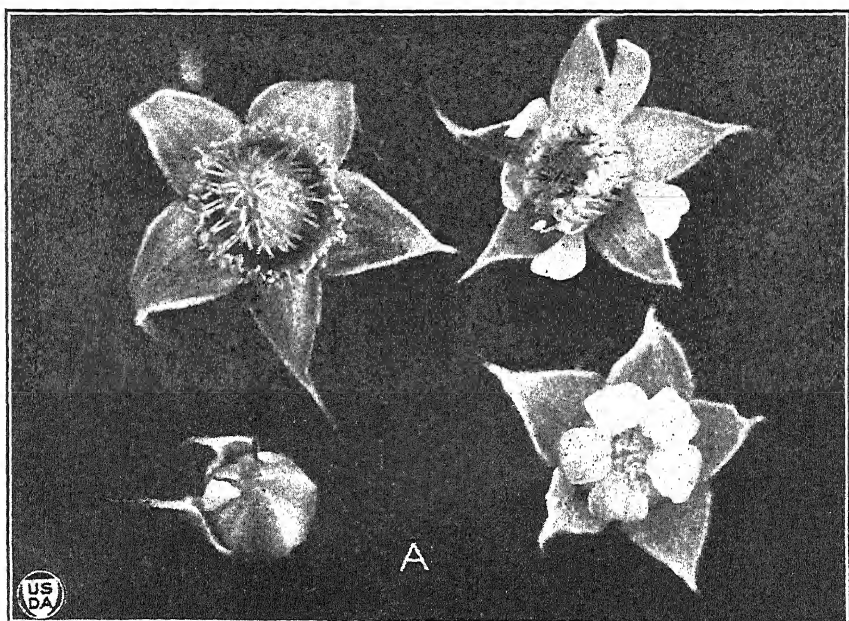
A.—Red raspberry buds and flowers. Pollen counts were made, for the most part, from anthers taken from buds at approximately the stage of development shown in the youngest bud.

B.—Photomicrograph of an anther from a La France×Ranere hybrid that was used for the study of the chromosome behavior in this triploid individual.

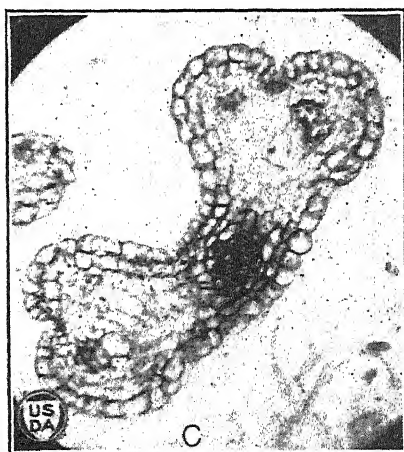
C.—Photomicrograph of anther from a sterile La Franc×Ranere hybrid.

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<sup>a</sup>The drawings of Plates 2 and 3 were made with a 2mm. Zeiss apochromatic lens, a No. 18 compensation ocular and a camera lucida. One exception, Plate 3, C, was made with a 1.7 lens and a No. 12 ocular. All drawings were made with the paper on the table level with the base of the microscope.



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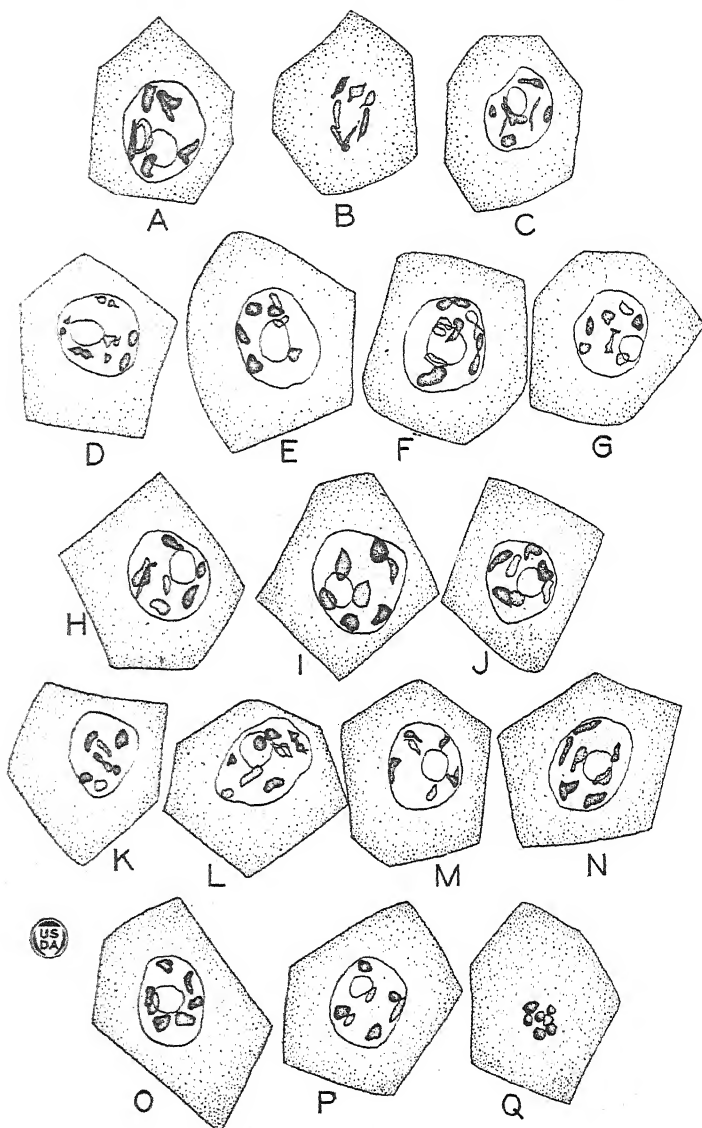


PLATE 2

Pollen-mother-cells of rubi at diakinesis:<sup>a</sup>

- A.—*Rubus strigosus*.
- B.—*Rubus idaeus*. Horticultural variety Lloyd George (early heterotypic metaphase).
- C.—*Rubus occidentalis*. Horticultural variety Cumberland, showing the two, long, slender chromosomes.
- D.—*Rubus idaeus*×*strigosus*. Horticultural variety Cuthbert (early prophase).
- E.—*Rubus occidentalis*×*idaeus*. Horticultural variety Gregg×Wisbeck.
- F.—*Rubus occidentalis*×*strigosus*. Horticultural variety Gregg×King.
- G.—*Rubus* (*occidentalis*×*idaeus*)×*strigosus*. Horticultural variety Royal×Newman No. 23.
- H.—*Rubus lasiostylus*.
- I.—*Rubus coreanus*.
- J.—*Rubus innominatus*.
- K.—*Rubus lasiostylus* variety *disygos*×*idaeus* horticultural variety Superlative.
- L.—*Rubus coreanus*×*strigosus* horticultural variety Newman.
- M.—*Rubus innominatus*×*idaeus* horticultural variety Superlative.
- N.—*Rubus innominatus*×(*idaeus*×*strigosus*) horticultural variety Cuthbert.
- O.—*Rubus phoenicolasius*.
- P.—*Rubus adenophorus*.
- Q.—*Rubus mesogaeus*.

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<sup>a</sup> Drawings reduced one-half.

PLATE 3

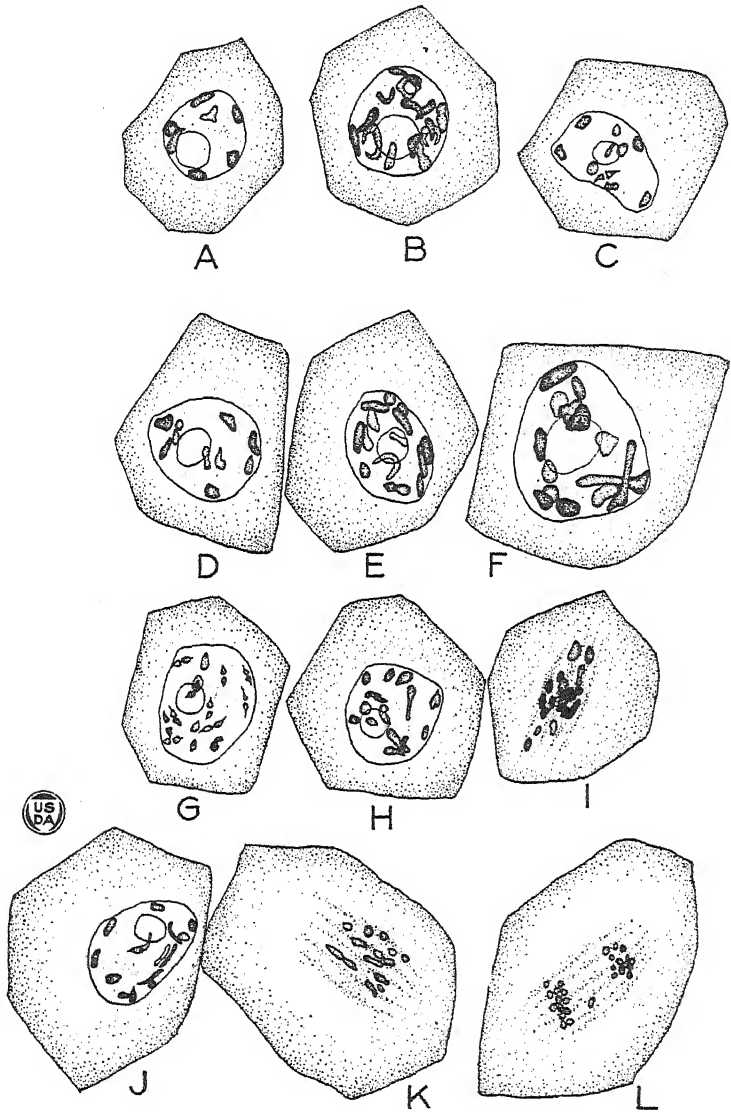
Pollen-mother-cells of some polyploidous rubi:<sup>a</sup>

- A.—*Rubus strigosus*. Horticultural variety Ranere, diploid.
- B.—*Rubus idaeus* (?). Horticultural variety La France, tetraploid.
- C.—*Rubus idaeus* (?) × *strigosus*. Horticultural variety La France × Ranere, triploid.
- D.—*Rubus strigosus* × *idaeus*, (?). Horticultural variety Erskine, triploid.
- E.—A tetraploid *Rubus*, Merveille de Rouge.
- F.—A tetraploid *Rubus*, Merveille de Quatre Saisons Rouge.
- G, H and I.—Three stages in the heterotypic division of Surpasse Merveille a blanc.
- J, K and L.—Three stages in the heterotypic division of the Ness, a tetraploid raspberry-blackberry hybrid.

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<sup>a</sup> Drawings reduced one-half.







# AECIDIOSPORE DISCHARGE AS RELATED TO THE CHARACTER OF THE SPORE WALL<sup>1</sup>

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During the prosecution of cytological studies on species of rust, the writer has made some observations on the development of their aecidia and on the origin and discharge of aecidiospores. The formation of germ pores and the concomitant development of structures which play an important rôle in spore discharge from the aecidium of *Gymnosporangium myricatum* (Schw.) Fromme are briefly described in this paper. Attention is also directed to important differences obtaining with respect to these features in the orange-rusts of Rubus.

The mechanics involved in the discharge of spores by such fungi as *Pilobolus* have always been a fascinating subject for investigation. More recently Buller (1)<sup>2</sup> has added greatly to our knowledge of spore discharge by the Hymenomycetes, and has pointed out that in the common mushroom there is a very delicate adjustment of organs to insure the unobstructed fall of the spores between the gills as they come to maturity. The basidiospore is started off along its sporobolic trajectory with an initial horizontal velocity imparted it by the force of some miniature explosion, as it were. The actual mechanics of the process still remain a mystery. It is a well-known fact that the sporidia of *Gymnosporangium* are frequently set free from the promycelium with enough force to carry them well beyond the other promycelia on the telial horns. Dietel (3) has shown by measurements that sporidia of certain rusts are discharged from sterigmata with sufficient violence to carry them out horizontally nearly a millimeter.

Observations that have been made on the part played by the peristomal teeth of moss capsules or by the segments of the "peridium" of the Geasters in spore discharge have probably led us to assume that the hygroscopic action of peridial cells of the roestelia type serves as much to check the scattering of spores during unfavorable weather as it does to dislodge them.

In attempting to make stereoscopic photographs of the beautiful aecidia of *G. myricatum* with a binocular outfit, the writer (5) was not very successful in obtaining clear pictures of the spores in the cups, because the group of spores brought into focus generally disappeared before the completion of the exposure. On examining aecidia on freshly gathered leaves with a microscope of fairly low power, it was noticed that the spores were being shot out of the cups one by one with considerable force like popped corn jumping out of the popper. When recently matured aecidia were dissected out of the leaf and placed on slides in damp chambers overnight, it was found that a fan-shaped spore print

<sup>1</sup> Received for publication Feb. 17, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 736

had been formed, spreading away from the mouth of the cup (fig. 1, A, B). These spores, mounted in water, appeared not to differ from ordinary aecidiospores, but examined dry directly on the slide on which they had been discharged, it was apparent that some of the spores had small spherical bodies attached to their walls. Many such granules lying scattered about over the field covered were at first thought to be particles of dust, but on going back to aecidia on fresh leaves and looking down into the aecidium cups it was found that the spores showed these bodies on their surfaces before being discharged. By carefully dislodging the bodies from the spores in dry mounts it became evident, as it had not been in liquid mounts, that some of them were located directly over the germ pores and that probably each germ pore originally was capped by one of these globules. The part that they might play in spore discharge became clear from a study of their development.

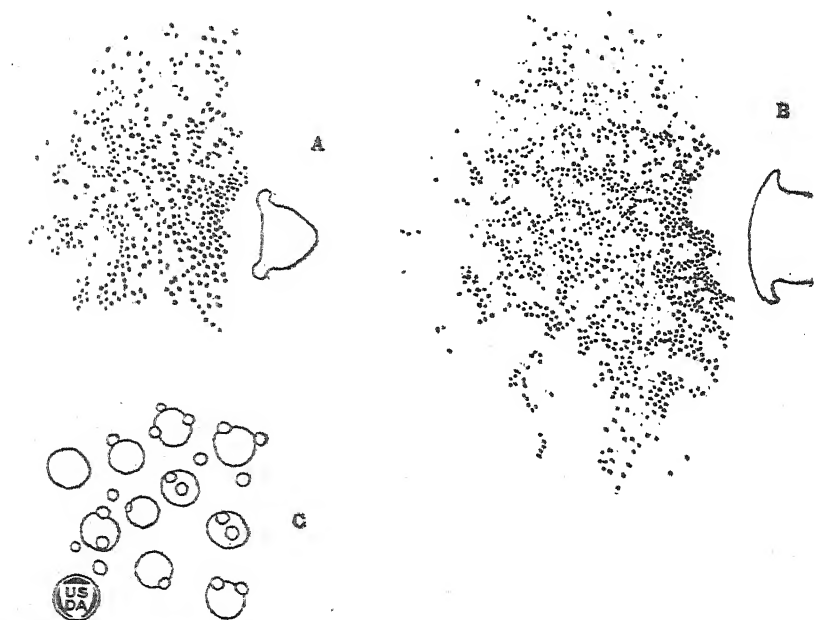


FIG. 1.—*Gymnosporangium myricatum*. A, B: Spore prints formed after dissecting out an aecidium, and laying it down on a glass slide in a damp chamber. The print was photographed and the diagram made from the photograph. Some of the spores were shot out distances equal to about 100 times the diameter of the spore. They might have been thrown farther if the aecidium had been placed somewhat above the glass support. C: Outline of spores showing comparative sizes and numbers of plugs that still remained attached to the spores after their flight.

Material for a cytological study of the bayberry rust was obtained by cutting out very small pieces of leaf or stem bearing young aecidia and fixing them in Flemming's fluid. The triple stain differentiates very well the structures with which we are particularly concerned.

Between the lowest spore cell being cut off from the basal cell and the outermost spore which is fully mature and is about to be discharged, there exists a number of spores in all stages of development (fig. 2). These spore chains are clearly prismatic in cross-section because they are under strong pressure, not only from the sides but from below as well, and the individual spores and intercalary cells are more or less angular in outline. Spores in adjacent chains are cut off at different

levels so that no two spores have exactly the same shape as they exist under these pressures. Only one layer can be made out in the wall of the youngest spore in the chain. Beginning with about the fourth spore from the base, differentiation of the wall into three distinct layers can be observed. The outer and inner layers are very thin, while the middle layer is somewhat thickened. Pore formation begins at about this stage. All of the spore wall takes a deep orange G stain except that region where the pore will later be found. This part becomes considerably thickened, remains slightly separated from the rest of the "exospore" and takes a brilliant gentian violet stain; the older the spore the larger and more easily distinguished becomes the violet-stained portion, which for convenience may be referred to as a "plug." It is very much flattened (fig. 3); its thickness is only about half its diameter. Viewed edgewise it appears to be made up of striae similar to the lines in the walls of the peridial cells. At least it is not homogeneous in its structure. Viewed flatwise it seems to be composed of wedge-shaped segments placed edge to edge. The prevailing shape of the fully formed plug is that of a basin or a hatter's block (fig. 3, B). The writer's conception of the mechanics of spore discharge follows.

The aecidium is slightly contracted where it comes in contact with the lower epidermis of the leaf, or if not, the effect is the same because the epidermis offers more resistance to the forces of expansion developed by the growing sorus than do the cells of the mesophyll. The spore walls are highly elastic so that at the spot opposite each

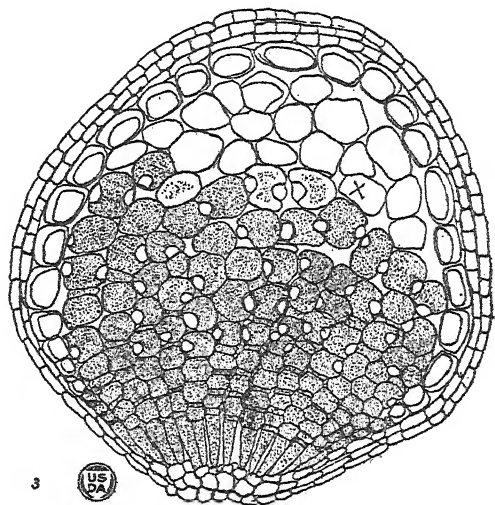


FIG. 2.—*G. myricatum*. Section of young aecidium. (See text for further explanation.)

plug the adjacent wall is much indented (fig. 3, A). As the maturing spores are being pushed upward new adjustments are brought about as the spores slide by one another over the separating plugs. While the shape of a spore under pressure is very irregular, it loses its angularity as soon as it is forced past the level of the epidermis so that these confining pressures are removed. The parts indented by the plugs spring back into place. The reaction of the walls of other spores touching this one is such as to violently expel the spore from the cup.

A section of a young aecidium is shown in figure 2, the layers of cells surrounding the aecidium being shown diagrammatically. The outlines of peridial cells, spores, and pore plugs were traced with the aid of a camera lucida. The unshaded spores in the upper part of the aecidium have no cytoplasmic contents. They are degenerating and by so doing may supply mucilaginous substances which by swelling assist in rupturing the

epidermis. The shading in this figure is simply to indicate cytoplasmic contents. The open spaces shown in this section are clearly due to shrinkage in fixation. If theaecidium were open and the degenerated spores cleared away, the spore just below X would be in a position to be shot out with considerable force.

Every spore is at first interlocked with its neighbors through the plugs indenting their walls and because the spore walls are rough or finely warted. At first the system only gradually moves out of the state of equilibrium. New adjustments are continually taking place until one spore is forced into the critical position necessary before it can be set free.

The correctness of the above explanation of the mechanism by means of which the aecidiospores of *G. myricatum* are ejected from the sori with such velocity can be demonstrated by the use of tennis balls and marbles. Confine the balls under pressure from all sides, except from

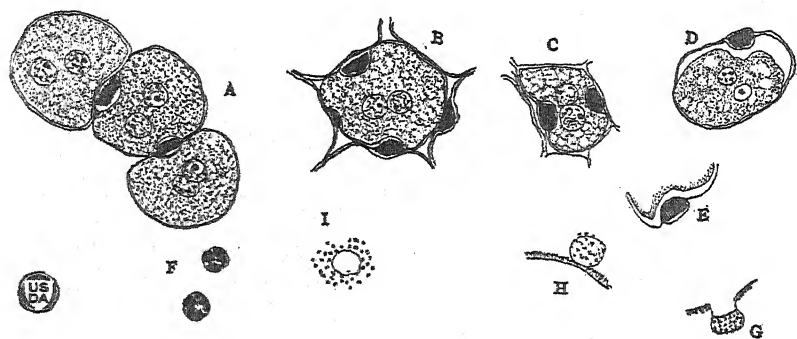


FIG. 3.—*G. myricatum*. A: Section of three adjacent spores showing pore plugs as they lie in the spaces which will become the germ pores. Note that the wall of the adjacent spore is indented in each case, and that the plug is covered on the outer and inner sides by very thin membranes. B: Section of a young spore parallel to the surface of the leaf. Two pore plugs and the edge of a third show in the section; one plug in an adjacent spore. C: Section of young spore with two large plugs. The indentations are due to side pressures and not to shrinkage. D, E show shrinkage of spore contents away from the wall. Material preserved in alcohol several years before imbedding. F: Surface view of pore plugs, showing that these bodies do not have a homogeneous structure. G, H: Position of pore plugs in dry mounts from herbarium specimens. I: Germ spore as it appears after dislodging the pore plugs

above, with marbles thrust in between them where they touch. By gradually increasing the pressure from the sides the balls become more and more compressed but remain in position. Now if pressure is applied to one of the balls from below it gradually moves up until the resultant of forces is such as to overcome the friction between the balls and between them and the marbles, and the ball will be liberated and suddenly shot upward. If the convexed bottom of a well chosen tin pan is pressed inward it will remain concave until set in motion by a slight pressure, or by warming the pan. The bottom moves out slowly at first until the critical position is reached, when it will suddenly complete the return with much noise. By placing two such pans together, bottom to bottom, one will be thrown upward violently, especially if the two bottoms in contact suddenly become convexed at the same time. The conidium of *Empusa grylli* is thrown off violently on the same principle. The two elastic membranes between the spore and its support suddenly become convexed. In this case the little columella may in reality function the same as the pore plug does in the *Myrica* rust, namely, as a protuberance against which the elastic membrane of the spore can react all the more

strongly. Similarly, a tennis ball compressed over a marble on a table will be thrown farther upward when the confining pressure is suddenly removed than it will be if compressed against the table alone with the same force, and then released.

Very little work has been done on aecidiospore discharge, so that it is impossible to explain the necessity for any special device for insuring that the spores be ejected forcibly from the aecidium as soon as they mature. The aecidiospores of a great number of rusts are provided with germ pores and it is not unlikely that in some of the species the spores are thrown out with considerable velocity. It is conceivable that in some cases this would be of decided advantage to the rust. In the rust on *Myrica* most of the aecidia develop on the under side of the leaves (5). If the spores dropped out of the cup as soon as mature, they could be borne away by the wind just as well as if they had been shot out a short distance. On the other hand, these spores are rather short-lived and as they are somewhat waxy they would tend to cling or mass together in the cup and fall out in clumps as do the spores of the short-cycled orange-rust of *Rubus* (4). The aecidiospores of this blackberry rust develop sporidia on promycelia as they germinate, functioning as teleutospores. It is an unreported fact that these spores frequently germinate during wet weather or on warm dewy nights as they hang together in masses on the under side of the leaves. The spores are particularly fitted to function in this way by developing a waxy coating which holds them together. Sporidia developed on the under side of the leaves a few feet from the ground would be much more likely to be borne away by air currents than would the heavy aecidiospores themselves.

The aecidiospores from roesteliae of other *Gymnosporangia* are certainly not discharged with violence. The writer's attention has not been attracted to any such persistent plugs developed in pore formation in roesteliae examined. In *G. clavariaeforme* where the germ pores are large and distinct from the first, the method of pore formation approaches that of pollen grains of geranium as described by Strasburger. In place of a thickened lid covering the pore, a granular substance is extruded which at first has the shape of a plug. It takes the orange G faintly and connects with the spore contents. The outer portion of the extruded mass is certainly not very different from the substance forming the thickenings on the exospore, but as the spore approaches maturity it is reabsorbed or withdrawn. The aecidiospores of this species, which are comparatively long-lived, collect in large quantities in the cancellate peridia and are allowed to escape through the hygroscopic action of the peridial cells.

A beautiful example of modification of the coating of the exospore to better adapt the aecidiospore for proper distribution is found in connection with the orange-rusts on blackberry. There can be no question that the two orange-rusts are very closely related. The preponderance of evidence is that the short-cycled rust has been or is being derived from the long-cycled form. The aecidiospores of the latter should be distributed widely so as to bring about numbers of sporophytic infections in new regions. This large and irregular *Gymnoconia* aecidium is of the caeoma type and as the spores are not provided with germ pore plugs it is difficult to imagine how the spores can be so confined under pressure as to be discharged at maturity in any way comparable to the method worked out in the case of the bayberry rust. Nevertheless when we examine blackberry leaves bearing the long-cycled orange-rust, we find that these aecidiospores

are also being discharged with considerable violence. If a Petri dish containing a thin layer of agar is inverted and a leaf bearing aecidia is placed in the cover so that the surface of the leaf which is covered with aecidia faces upward, it can be proved that the spores are frequently thrown vertically to a distance of 4 or 5 mm. or more. Many spores not hitting the agar, or if so, not sticking, fall back on the leaf or on the glass at some distance. Further study of the mechanics of spore discharge by the orange-rust will certainly prove interesting. These spores are not very waxy and tend to fall out of the sori soon after maturity, and they can stand a certain amount of drying. There is, however, enough wax on the exospore to insure that many of the spores as they fall will stick to the other leaves of the plant on which they were originally developed. By this compromise we find that teleutospores are commonly developed on the original host plant as well as on plants some distance away. The waxy coating is more highly developed on the aecidiospores of the short-cycled orange-rust, and as noted previously, this tends to hold the spores together in the sorus on the leaves well above the ground. Germinating in this position the resulting sporidia, well adapted for dispersal by air currents, begin their flight from an elevated position. The teleutospores of *Puccinia malvacearum* germinate in the sori as they hang on the living leaves, as soon as they are mature.

If one desires to infect a root shoot of a blackberry with the short-cycled orange-rust, he will be more successful if he sows sporidia on the shoot. If he sows aecidiospores he will be less successful; one reason for this may be that the sporidia which are to produce the infection are discharged from the promycelia with sufficient force to carry them away from the very place where they should come to rest if infection is to be obtained.

Not all of the aecidia of the bayberry rust are borne on the under side of the leaves. In many cases the ends of young branches and the fruit buds are covered with them so that the cups open out in all directions. This is especially true of the rust on the "sweet fern," *Comptonia*, where the burlike fruits and entire terminal parts of certain branches are attacked. The leaves bearing aecidia are also contorted and coiled into a form resembling that of a ram's horn (4). Such locations bring the aecidia into positions unfavorable for spore discharge unless the spores are started off with an initial impulse. Without entering into an extensive discussion of the formation of germ pores in other types of spores, it may be interesting to note that Strasburger and others have studied pore formation in the walls of pollen grains of several species of plants, and further that there are homologies and analogies between the pore plugs described above and germ pore lids in certain pollen grains.

Strasburger (8) in his well-known work on the structure and growth of cell membranes describes the development of the walls and germ pores of several different kinds of pollen. Two layers are differentiated; the outer layer is referred to as the exinium, or exine, which is commonly called the exospore. An inner layer Strasburger identifies by the terms intinium or intine (endospore). In the geranium (8) (fig. 27-38), thickening of the outer wall of the pollen grain does not occur at certain points. These thin places in the wall are the germ pores. Due to pressure from within, some of the cytoplasmic contents of the cell along with some of the endospore, are forced through the germ pores and form papillae. The granular contents become lined up in striae and stain



brown at first, then blue, with iodine. While in this condition the papillae superficially resemble very closely the striated germ pore plugs of the *Myrica* rust being considered here; they are of an entirely different origin and not to be compared, except as to location, with respect to the germ pores. The lids or caps which cover the germ pores in *Cucurbita* pollen (Strasburger (8), fig. 77-84) are more nearly analogous because the lids are developed out of the exospore layer. Had the caps developed beneath and not out of the original cell wall, they could be said to be homologous to the exospore thickenings of aecidiospores and of zygospores of the *Mucoraceae*.

Vuillemin (9) who studied the development of the walls of zygospores of *Sporodinia* and other *Mucoraceae*, recognizes five different layers or membranes. The innermost layer is composed of a granular matrix. The next part of the wall is referred to as the cartilaginous layer. This is bordered by a middle, thin membrane. The fourth layer is usually heavily carbonized and developed into characteristic warts or spines. Surrounding the young zygospore is the thin membrane, originally the primitive wall of the progametes.

Dangeard (2) features the two membranes which are easily separated. The exospore, warted and carbonized, originates as a separate cone-shaped thickening beneath the thin outer membrane. The endospore is the thick layer within which follows the undulations of the exospore. Lendner (6), Moreau (7), and others studying zygospore formation also show that in several species the warts or thickenings originate as separate cone or dome shaped structures in a matrix and by lateral extension fuse together to form the firm carbonized layer of the spore wall. Without lies the primitive membrane of the gametangia; within there remains a separable membrane of considerable thickness in contact with the cytoplasmic contents of the zygospore. Disregarding the outer thin layer of the young spore wall which is usually not to be distinguished in the mature spore, the two remaining layers are referred to as "exospore" and "endospore".

The pore plugs in the aecidiospore of the bayberry rust develop in the matrix or layer out of which the thick warty or echinulate exospore is formed but outside of this heavy wall, what the writer has interpreted as the thin primitive membrane of the spore wall can be seen if the spore cells are plasmolized. The inner membrane then also becomes visible.

As noted above, Dietel has shown that sporidia from certain teleutospores are sometimes projected 0.6 to 0.8 mm. The writer has made no attempt to learn just how far a spore can be thrown as it is discharged from the aecidium. The spore print (fig. 1) shows that every spore is forcibly expelled; there is always a clear space around the mouth of the cup where no spores have fallen. Not many spores are projected a distance of over one hundred times their diameter when an aecidium is laid directly on a glass. As the diameter of an aecidium is only a few times that of a spore, it can be seen that a spore has not long to fall before it hits the glass. Spores shot from aecidia growing horizontally on buds or coiled leaves would certainly be scattered much farther and more efficiently if they were given an initial horizontal velocity.

Germ pores of certain teleutospores are very commonly figured as being covered by very delicately bounded papillae. Structures like the papillae in the geranium pollen represent in part extruded cell sap of cell contents. The pore plugs are persistent morphological structures

which often cling to the spores very firmly. Such bodies can be found on the aecidiospores of the *Myrica* rust in any good herbarium specimen. Regardless of the actual mechanics of the discharge in this case, the spore in its flight no doubt follows closely the sporabolic curve described by Buller for the Basidiomycetes, so that beyond a certain and definite limit it probably makes little difference so far as the length of its horizontal flight is concerned, how high above the glass slide the aecidium is supported in the experiment referred to. The spore is shot off with a very great horizontal velocity which carries it almost straight out for a certain distance. Because of air resistance it then takes an abrupt turn and falls straight down unless carried away by currents.

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# HASTENING THE COLORATION OF LEMONS<sup>1</sup>

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## INTRODUCTION

Lemons are picked not only according to color, but also according to size. Thus all fruits that have become yellow on the tree are picked, and in addition those with a green color if they have reached a certain size as determined by a wire ring carried by the picker. As a result, when lemons reach the packing house a proportion, which varies with the field and with the season, is green in color. Such fruits are sorted into light green and dark green grades, and the yellow color is subsequently brought out by one of two general methods, the choice of method depending upon market conditions, that is, the time at which the house manager wishes to have the fruit ready for shipment.

If a delay in shipment is desirable, the green lemons are placed in storerooms, usually in a basement, at a temperature of from 50° to 55° F., with a humidity of about 80 per cent. The fruit becomes yellow in from 30 to 60 days.

If, however, the demand for fruit is brisk, the "forced coloring" method is used. This system has been much improved since its introduction many years ago. According to the original procedure, the fruit is placed in rooms or tents heated with kerosene stoves, with the result that the lemons turn yellow in one or two weeks. A source of humidity is often, although not always, provided to prevent shrinkage.

It was thought that the coloration was brought about by the temperature and humidity conditions in the heated rooms, but Sievers and True (17)<sup>2</sup> conclusively showed that the results were produced mainly by the gaseous combustion products from the kerosene stoves. Their experiments showed further that the gases did not lose their effectiveness by being conveyed from one room to another by pipes, using either forced or natural draft. Hence the more general method now in use consists in generating the kerosene-stove combustion products, hereafter referred to as "stove gas," in a separate building, called a generator room, and carrying the gases through conduits to the various fruit rooms.

Answers to a questionnaire sent to packing-house managers showed a general lack of agreement on most of the important details of operation. Uniformity in color and quality of fruit was not obtained, and it seemed impossible to fix upon any standardized procedure as being the one that gave the best results. This condition probably was due to the fact that no one knew what gas or gases in the sweat-room atmosphere caused the change. Since this seemed to be the critical question, experiments were started to determine if possible the identity of the gaseous constituent responsible for the coloration of the fruit.

<sup>1</sup> Received for publication, Jan. 23, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 768-769.

EFFECT OF GASES ON COLORATION OF LEMONS AS DETECTED BY  
STANDARD ANALYTICAL METHODS

## STOVE GAS

No analyses of the combustion products from kerosene stoves were found in the literature. Analysis of samples taken from the air above the burning flame showed: Carbon dioxid ( $\text{CO}_2$ ), 0.73 per cent, 0.63 per cent; oxygen ( $\text{O}_2$ ), 20.01 per cent, 19.94 per cent; no unsaturated gases; and carbon monoxid ( $\text{CO}$ ), 0.48 per cent, 0.05 per cent. Analysis of samples of ordinary air, using the same apparatus and reagents, gave: Carbon dioxid ( $\text{CO}_2$ ), 0.0 per cent, 0.04 per cent; oxygen ( $\text{O}_2$ ), 21.04 per cent, 21.11 per cent; no unsaturated gases; and no carbon monoxid ( $\text{CO}$ ). The oxygen percentages were, of course, too high, but no attempt was made to determine the cause. The data indicated that it was not feasible to search for the effective gas by ordinary gas-analytical methods.

## AUTOMOBILE EXHAUST GAS

Sievers and True (17) pointed out that the exhaust gas from automobile engines can color lemons. The composition of this gas has been very carefully determined (8, 2). Emphasis was laid upon completeness of combustion, however, because of its importance from the standpoint of fuel economy, and upon the quantity of carbon monoxid formed, because of its toxicity to man. The other gases present were not sorted out individually but were listed as heavy hydrocarbons, unsaturated gases, illuminants ( $\text{C}_n\text{H}_{2n}$ ), etc.

## SWEAT-ROOM ATMOSPHERE

S. A. Weirman, formerly of the laboratory of fruit and vegetable chemistry, United States Department of Agriculture, analyzed many samples of air taken from various sweat rooms in which fruit was being colored by kerosene stove gas. The quantities of carbon dioxid varied from 0.1 per cent to about 1.5 per cent, in one case reaching 4.9 per cent. The oxygen content was generally about 18.6 per cent. Carbon monoxid in small quantities was found in some samples. Less than one-third of the samples showed the presence of heavy hydrocarbons, the greatest quantity being 0.25 per cent.

## CARBON MONOXID AND CARBON DIOXID

The two gases among the combustion products positively identified by these methods are carbon monoxid and carbon dioxid. Carbon dioxid in a wide range of concentration was tried by Weirman, but it failed to produce the desired result. He found also that carbon monoxid in high concentration did not induce coloring. Later experiments, however, have shown that weaker concentrations are effective. Eight per cent of carbon monoxid caused coloration in about one week, even with gas that had been bubbled through bromin water to remove unsaturated hydrocarbons. However, 1 per cent of carbon monoxid was ineffective, only a slight change resulting, and one part of carbon monoxid in 1,000 parts of air gave no detectable change.

Therefore carbon monoxid is not the effective constituent, since concentrations of this gas sufficient to produce coloring would cause the death of

human beings in a few minutes. Such concentrations do not exist in commercial sweat rooms.

#### EFFECT OF GASES DETECTED BY SPECIAL METHODS

A stream of stove gas was bubbled through Pettenkofer tubes, some filled with 10 per cent potassium hydroxid solution and some with distilled water. After about three days the solutions were poured out and the liquids were tested for various substances.

A portion of the aqueous solution obtained in this way was tested for formaldehyde by the Rimini test (15, p. 85). The result was positive. Indeed, formaldehyde caused lemons to turn yellow, but the color was too brassy, and sunken spots formed in the rind wherever drops of vapor condensed. A similar result was obtained with acetaldehyde.

The solution of potassium hydroxid through which the stove gas had bubbled was tested for nitric oxid by the method described by Dennis (5, p. 218). A distinctly positive test was given. Nitric oxid, generated from sheet copper, potassium nitrate, and concentrated sulphuric acid were applied to green lemons in desiccators. None of the lemons turned yellow, but some of them showed surface injuries.

The water and potassium hydroxid absorptions were also tested for the presence of phenols by Scott's method (16). A positive test was obtained. The vapor from phenol turned green lemons yellow in about six days and no blemishes were caused. However, it did not seem possible to make any practical application of this fact.

Stove gas was bubbled through Pettenkofer tubes containing solutions of ammoniacal and of neutral silver nitrate. A small quantity of a precipitate, probably silver acetylide, was formed. On adding acid, this decomposed, giving off a gas with an odor of acetylene. Acetylene from a commercial cylinder at a concentration of 1 to 1,000 was effective in bringing about coloration. Since commercial acetylene is said to contain traces of other gases, however, acetylene of greater purity was prepared by treating ethylene dibromid with alcoholic potash (14, p. 84). The gas generated in this way caused no change in the coloration of lemons after exposure for seven days to concentrations of 1 to 100, 1 to 1,000, and 1 to 50,000. Hence the coloration obtained by the use of commercial acetylene is thought to have been caused by the impurities present and not by the acetylene itself.

#### REMOVAL OF EFFECTIVE CONSTITUENT FROM STOVE GAS BY ABSORBENTS

Since the effective constituent seemed to be present in the stove gas in low concentrations only, an effort was made to absorb this trace by reagents. In the apparatus devised for this work (fig. 1), a current of gas from the stove was drawn through the system by reduced pressure at S, the reduction being equivalent to about 7 inches of water below atmospheric. By a stopcock at C, bubbles from 3 to 4 mm. in diameter were made to follow one another in rapid succession up the tube in which they were washed by the absorbing liquid. From 7 to 10 seconds were required for a bubble to travel the length of the tube.

It can not be stated that complete absorption took place, since, for the complete removal of a gas by a liquid, shaking for several minutes is often recommended. When solid-absorbing reagents were used

they were placed in straight glass tubes, 4 to 5 feet long and  $\frac{3}{4}$  to 1 inch in diameter. If necessary, gas-washing bottles were inserted at *W**B*, the first filled with the proper liquid to remove any vapors carried over the absorption tubes and the second to keep the gas saturated with water vapor. The residual gas then passed into the desiccators containing green lemons. In order to permit a conclusion as to the effect of the absorbing substances, two control lots were provided. One was a similar lot of fruit treated with a stream of unabsorbed stove gas, and the other was a desiccator of green fruit aerated each day with outdoor air, receiving no gaseous treatment.

The following solid reagents did not remove the effective constituents from the stove gas: Granular calcium chlorid, soda lime, activated charcoal, and silica gel. Complete removal, however, was obtained by means of hopcalite (9, *p.* 110). Stove gas dried by calcium chlorid and passed through a small tube containing about 25 gm. of granular hopcalite was no longer capable of inducing coloration of green lemons. This reagent is noted for its ability to oxidize carbon monoxid, but the firm manufacturing it states that not only carbon monoxid but many other oxidizable gases are acted upon by it.

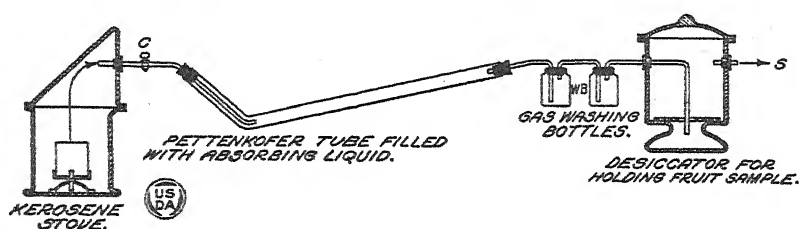


Fig. 1.—Apparatus for removal of constituents from stove gas.

The following liquid-absorbing reagents did not completely remove the effective constituent from stove gas: Water, concentrated sulphuric acid, 50 per cent potassium hydroxid, 0.75 per cent, potassium permanganate, 10 per cent silver nitrate, and saturated aqueous solution of mercuric acetate.

When the stove gas was bubbled through saturated bromin water and when the bromin vapor was removed by dilute sodium hydroxid, however, the residual gas did not color green lemons. This result suggested the desirability of testing the effect of unsaturated gases such as ethylene.

#### PRELIMINARY LABORATORY EXPERIMENTS WITH ETHYLENE IN LOW CONCENTRATIONS

In the preliminary experiments ethylene was generated by dropping ethyl alcohol upon phosphoric acid as described by Doubt (6). When it was found that ethylene could be bought as a compressed gas in steel cylinders, most of the experiments were conducted with gas from this source. An analysis of gas from the cylinder showed that 97.7 per cent by volume was absorbed by bromin water.

For testing the effect upon green fruit of ethylene at low concentrations, gas from the cylinder was diluted with air to make ethylene-air mixtures of 5 per cent, 1 per cent, 0.1 per cent, 0.05 per cent, and 0.01 per cent. Green fruits, the number varying from 6 to 24, were placed in

large glass or metal vessels with a capacity of from 2,500 to 50,000 cc. Allowance being made for the volume occupied by the fruit, gas from the stock ethylene-air mixtures was added from a gas burette in sufficient quantities (usually 10 to 50 cc.) to give the desired concentration of ethylene in the atmosphere surrounding the fruit. This was done once or twice daily. The vessels containing the fruit were aerated by outdoor air for purposes of ventilation before the gas was added. Control lots consisting of fruit receiving treatment with outdoor air only were provided in each experiment. The following proportions by volume of ethylene to air were tried: 1 to 1,000; 1 to 5,000; 1 to 10,000; 1 to 50,000; 1 to 100,000; 1 to 200,000; 1 to 250,000; 1 to 500,000; 1 to 1,000,000; 1 to 2,000,000; and 1 to 5,000,000.

In all cases coloration of green lemons resulted. The effect was not in proportion to the concentration. Concentrations from 1 to 1,000 to 1 to 200,000 produced approximately the same effects, coloring the lemons in from 5 to 8 days. Concentrations down to 1 to 2,000,000 were somewhat less effective, requiring 1 or 2 days longer. Concentrations of 1 to 5,000,000 required about 14 days, indicating that this concentration represents the highest dilution at which coloration is markedly hastened.

The effect of ethylene was further checked in one case by bubbling the gas-air mixture through bromin water before permitting it to come in contact with the fruit. In this case coloration did not result.

#### EXPERIMENTS WITH ETHYLENE UNDER COMMERCIAL CONDITIONS

##### EFFECTS OF ETHYLENE AND OF STOVE GAS

Forty-eight boxes of dark-green lemons, divided into two equal lots, were placed in two large sweat rooms of commercial size (about 6,600 cubic feet), provided by a California citrus association. In one room a kerosene stove was burned throughout the experiment in a manner identical with present commercial practice. Into the other ethylene was measured out from the cylinder through a gas meter. Twenty-five applications of the gas were made at intervals of six hours, a total of 48.55 cubic feet being liberated into the room. At first the air was stirred with an electric fan to distribute the ethylene, but later this was discontinued, being unnecessary. The temperatures and humidities of the two rooms were kept the same by close attention and hand regulation, heat equal to that formed by the kerosene stove being provided for the second room by means of hot-water coils near the walls of the room. The temperature ranged from 60° to 65° F., and the humidity from 80 to 90 per cent. The fruit in both rooms colored in eight days and no difference in the two lots could be noted, either with respect to the color developed or to the commercial quality of the finished product.

##### METHOD OF MEASURING AND APPLYING THE GAS

It was neither convenient nor accurate to measure the gas from the cylinder with a gas meter, but the apparatus shown in figure 2 was satisfactory in every respect for this purpose. *A* is the cylinder of compressed ethylene, and *B* is an empty cylinder capable of withstanding pressures up to 50 pounds. *B* serves as a "measuring cylinder." *C* is a pressure gauge, reading in pounds per square inch. It is desirable to

place a pet cock between the gauge and the measuring tank in order to protect the gauge from the sudden increase in pressure when the gas is released from the ethylene cylinder. *D* is the rubber hose outlet. To measure gas with the apparatus, valve *E* is closed and valve *F* is opened. As ethylene is forced over into *B*, the reading on the pressure gauge rises. If *F* is now closed and *E* opened, gas is released from the cylinder and the pressure-gauge reading falls. After a few such operations *B*, of course, becomes filled with pure ethylene gas at atmospheric pressure. Gauge readings were calibrated by collecting and measuring the released gas. In this particular case a reading of 10 pounds was equivalent to 1 cubic foot of gas.

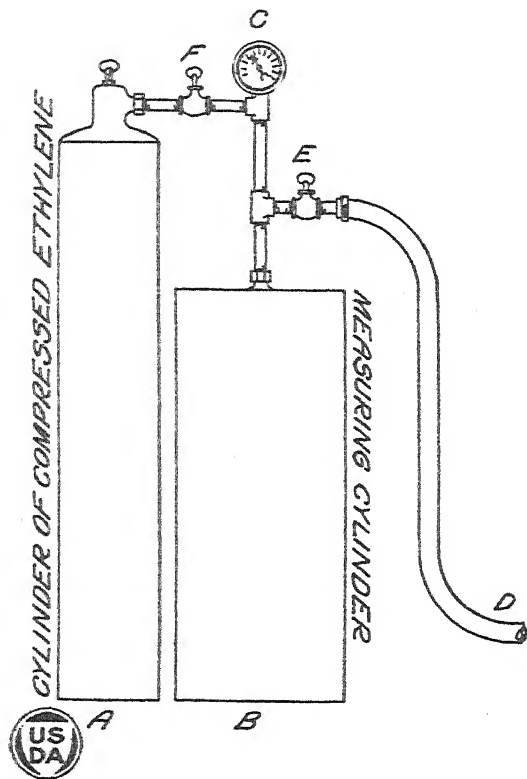


Fig. 2.—Apparatus for measuring ethylene from cylinder.

by compressed air, which gave a very fine mist. Seventy-two boxes of green lemons, divided into three equal lots, were placed in the rooms. Every six hours 1 cubic foot of ethylene from the cylinder was measured out into each room.

The fruit in the room at 82° F. colored up in 4 days, that at 68° F. in 5 days, and that at 57° F. in 9½ days. In all lots, the color was good. In the room at 57° F. the buttons were firmer and less discolored than those of the fruit in the other rooms.

The effect of higher and lower temperature was tested in the laboratory with small quantities of fruit. At a temperature of 45° F. and with ethylene of a 1 to 1,000 concentration, no development of color took

The measuring apparatus was mounted on a wheel truck for convenience in gassing the various rooms or experimental lots of fruit.

#### EFFECT OF TEMPERATURE ON RATE OF COLORATION

Three of the large rooms previously mentioned were used in this experiment, the first being kept at 78° to 83° F. (average, 82° F.), the second at 64° to 69° F. (average, 68° F.), and the third at 52° to 60° F. (average, 57° F.). Heat was furnished by an automatic hot-water heater in the hallway in the basement. The hot water circulated in pipes attached to the side walls of the rooms and the temperature was regulated by a valve in the hot-water pipe line. Moisture for humidifying the rooms was produced by means of large atomizers operated



place in 10 days. In an incubator at 93° F. and with ethylene of a 1 to 10,000 concentration, coloration took place in 9 days, but the color was not as well developed as with a similar lot of fruit held at a temperature of 68° to 72° F. Thus a low temperature practically inhibited coloration and a high temperature reduced the rate.

#### EFFECT OF REDUCING THE NUMBER OF GAS APPLICATIONS

In the first experiments in commercial rooms, gas applications were made four times a day. More than two a day would be inconvenient in practical work. Accordingly, about half a carload of green lemons, representing boxes of fruit from several groves, were placed in a sweat room and one cubic foot of ethylene was released into the room twice daily. For the purpose of ventilating the fruit, once daily, just before a gas application, the ventilator and doors were opened for one hour, and air was drawn into the room by means of an electric fan. The temperature of the room during the experiment was 68° to 73° F. (average, 70°). The humidity was 75 to 90 per cent (average, 81 per cent).

The different lots of fruit in the room colored at different rates. The first lot was removed at the end of 4 days, and other lots at the end of 6, 7, and 8 days. One lot was not completely colored at the end of 10 days. The color and condition of the fruit at the end of the experiment were satisfactory. It is not known what concentrations of ethylene existed in the room during the experimental period, since no method of estimating ethylene in such low concentrations was found. After liberation of the gas diffusion losses at once began. The extent of these losses could not be determined, but data in an unsigned article (1) regarding air exchange in a closed room indicate that they would be large. The experiment, however, showed that two applications of gas a day were sufficient to induce coloring.

#### COLORING FRUIT IN TENTS

Fruit was not placed in a special room, but the boxes were stacked in an open hallway and covered with two thicknesses of ordinary cotton tent canvas, such as is used in fumigating with hydrocyanic acid gas. Four times daily the outlet hose from the cylinder was pushed under the edge of the tent and ethylene was admitted to make a concentration of ethylene amounting to 1 part in 250. This lot was exposed to the temperature of the outdoor air and varied from 50° to 76° F., with an average of 58°.

At the end of 10 days the fruit was full yellow. The color was slightly paler and more attractive than that of similar fruit colored in regular sweat rooms. It appeared that the tent covering retained the gas well enough to permit coloring to proceed in a satisfactory manner.

#### CONDITIONS PREVENTING OR RETARDING RATE OF COLORING

##### HIGH CONCENTRATIONS OF ETHYLENE

Large bottles were filled with a gas mixture consisting of 80 per cent ethylene and 20 per cent oxygen by volume. By water displacement, a supply of gas was pushed over once each day into desiccators containing green lemons. For comparison, a second lot of fruit was treated in a similar way, except that the ethylene concentration was 1 to 1,000. A third lot received no gaseous treatment but was aerated with outdoor air each day. At the end of 7 days, the lemons in the third (control)

lot were still green, those in the second lot (ethylene 1 to 1,000) were yellow, and those in the first lot (ethylene 80 per cent) were slightly more yellow than the controls but not more than one-eighth colored, the buttons being firm on all but one. High concentrations of ethylene appear to retard the rate of coloring.

#### LACK OF OXYGEN

Pure nitrogen, generated from ammonium chlorid and sodium nitrite, was collected over water in a 19-liter bottle. To this nitrogen ethylene was added to make a concentration of 0.5 per cent by volume. By water displacement this gaseous mixture was pushed over into a desiccator containing green lemons. In order to remove the oxygen as completely as possible, the bottom of the desiccator was covered with alkaline pyrogallate, which was also placed in wash bottles through which the gas was bubbled before entering the desiccator. For comparison, another lot of fruit was treated in an exactly similar manner except that outdoor air was used instead of nitrogen and water was substituted for alkaline pyrogallate. Fresh quantities of gas were pushed over into the desiccators each day. The control lot was colored yellow at the end of eight days but the lot receiving no oxygen remained green. A duplicate experiment, using 1 per cent of ethylene, gave similar results. Oxygen appears to be necessary for coloring.

#### EFFECT OF ETHYLENE AND OF STOVE GAS UPON RESPIRATION OF LEMONS

The experiments showing that coloration was prevented or interfered with by high and low temperatures and by high concentrations of ethylene, that it was favored by intermediate temperatures, and required oxygen, indicated that coloring was hastened by conditions that were favorable to the life processes of the fruit. Furthermore, whenever ethylene or stove gas was used, coloration was accompanied by the loss or loosening of the buttons (calyx and a portion of the clipped fruit stem). The cells at the absciss layer were greatly increased in size, that is, growth and enlargement had taken place. In many cases there was an extrusion of tissue just below the buttons, a condition similar to that described by Doubt (6) and others. If ethylene and stove gas induce coloration by a stimulation of the growth of the cells, or by increasing their life activity, the respiration of the treated fruit should be increased.

Ethylene in concentrations of 1 to 1,000 and 1 to 1,000,000 markedly increased the carbon dioxid output, the percentage increases ranging from 100 per cent to about 250 per cent.<sup>3</sup> A comparison was made of the effect of ethylene and of stove gas upon the respiration of lemons. Six lemons, placed in each of six desiccators, received the following treatment:

*Lots A and B.*—A current of stove gas was drawn through the desiccators for six hours each day. The desiccators were then closed and placed in an incubator at 25° C. until half past 8 o'clock the following morning.

*Lot C.*—Ethylene to make a concentration of 1 to 10,000 was added twice each day, after thorough aeration in outdoor air. The desiccator was then placed in the incubator simultaneously with lots A and B.

<sup>3</sup> DENNY, F. E. THE EFFECT OF ETHYLENE UPON THE RESPIRATION OF LEMONS. *In* Bot. Gaz. Not yet published.

*Lot D.*—Same treatment as lot C, except that the concentration of ethylene was 1 to 200,000.

*Lots E and F.*—These control lots received no gaseous treatment but in other respects were handled in the same way as lots C and D.

Every other day the fruit was removed from the desiccators and aerated for one-half hour. The air in the desiccators was forced out by filling them with water, the fruit was replaced, and a current of air was aspirated through the system for 1½ hours, the carbon dioxide being absorbed in barium hydroxid solution. From the data thus obtained the number of milligrams of carbon dioxide per kilogram per hour was calculated. The results are shown in Table I and graphically in figure 3.

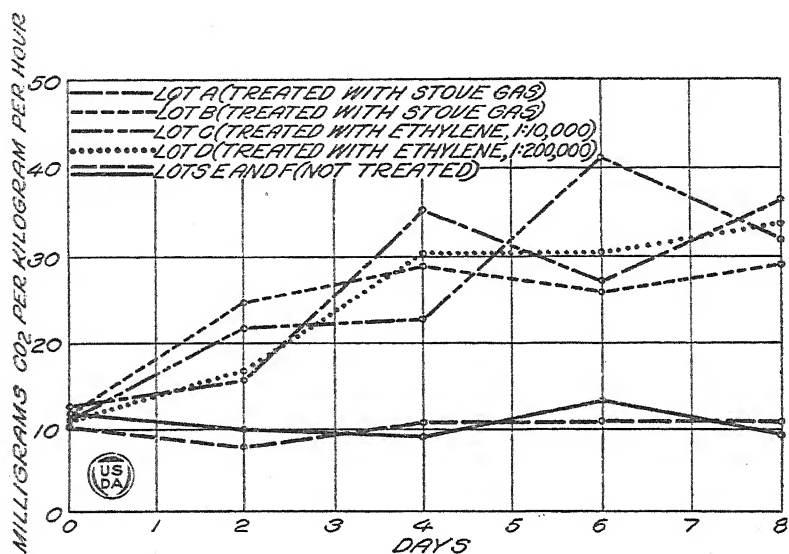


FIG. 3.—Effect of stove gas and of ethylene on respiration of lemons.

The respiration was increased by both ethylene and stove gas, the percentage increase being from 150 to 250 per cent. No essential difference between ethylene and stove gas with respect to the increase in respiration was shown. Further experiments are needed to decide this point.

TABLE I.—Effect of ethylene and of stove gas upon respiration of lemons

Lot No.	Weight of fresh fruit.	Treatment.	Rate of respiration.				
			Start.	Second day.	Fourth day.	Sixth day.	Eighth day.
	<i>Gm.</i>		<i>Milligrams of carbon dioxide per kilogram per hour.</i>				
A	707	Stove gas.....	12.3	15.1	34.9	26.4	35.8
B	699	.....do.....	11.2	24.3	28.6	24.8	25.8
C	698	Ethylene, 1 to 10,000....	10.5	21.0	22.0	41.1	30.6
D	649	Ethylene, 1 to 200,000....	11.3	15.4	29.8	28.8	32.9
E	658	Control.....	10.1	7.1	10.1	10.1	10.1
F	693	.....do.....	11.5	9.1	8.7	12.5	9.6

IS ETHYLENE PRESENT IN THE COMBUSTION PRODUCTS OF  
KEROSENE STOVES?

No delicate specific qualitative test for ethylene in a mixture of gases was found. An attempt was made to isolate ethylene from stove gas by bubbling it through bromin water. This was done for periods of 6 to 10 days, about seven hours a day, on three different occasions, the absorbing tube being surrounded by ice in one case. No detectable quantity of bromid was obtained. It is believed, however, that ethylene, if present in stove gas, occurs there only in traces. If so, it would be difficult to detect it in this way. Thus, on the basis that 15 gm. of ethylene dibromid would be needed for the identification tests, if ethylene were present at a concentration of 1 to 1,000, it would be necessary to bubble stove gas through bromin for 24 hours a day for about 18½ days. At a concentration of 1 to 10,000 about six months would be required.

Aside from the fact that ethylene and stove gas produce similar effects upon green fruit, there are other reasons for believing that ethylene is present in stove gas. Ethylene has been found among gases that are produced in the cracking of petroleum (11). Denig (4) obtained ethylene as one of the products of the decomposition of kerosene by heat. According to Eldred and Mersereau (7), ethylene is produced by heating kerosene vapor under certain conditions. Lewes (10) states that ethylene is "found as one of the products in nearly all cases where organic compounds are subjected to distillation at high temperatures." A further fact pointing to the same conclusion was obtained when, in attempting to remove oxygen from stove gas by phosphorus, the phosphorus would not burn. White (19) states that "a fraction of a tenth of a per cent of ethylene will completely prevent the reaction between phosphorus and oxygen."

## ETHYLENE FROM THE PRACTICAL STANDPOINT

## EXPLOSIVE AND ANESTHETIC PROPERTIES

The sources, chemical behavior, physical constants, and important commercial uses of ethylene are discussed fully by Curme (3) and by Malisoff and Egloff (13). In connection with the experiments here reported two properties of the gas need to be discussed:

(a) When ethylene is mixed with air in proportions above 3 per cent by volume and below 30 per cent by volume, the mixture may be exploded by ignition. In liberating ethylene from the cylinder into a room containing fruit to be colored, however, explosive concentrations are not reached. To reach such proportions in a room of carload capacity, say 3,500 cubic feet, it would be necessary to liberate about 100 cubic feet of ethylene, or about one-third of the entire contents of a full cylinder. The lowest explosive concentration is at least 100 times as strong as the strongest application of gas needed for successful coloration.

(b) Luckhardt and Carter (12) have found that animals, including human beings, can be anesthetized by ethylene at a concentration of 80 per cent ethylene and 20 per cent oxygen. The effectiveness, however, decreases rapidly with decreasing concentration. Thus Smith and Hoskins (18) were unable to anesthetize or injure a mouse with 72.5 per cent ethylene and 27.5 per cent oxygen by exposure for one hour. The writer has freely breathed the pure gas as it comes from the cylinder and

has remained in the room for at least an hour after liberating a charge of gas and at no time has he noted any physiological effects. The concentrations necessary to produce anesthesia are about 800 to 8,000 times as strong as those recommended for coloring lemons.

#### COST OF ETHYLENE TREATMENTS

One cylinder of ethylene holds about 320 cubic feet of the gas at ordinary pressure and temperature. At present prices, the gas costs about  $3\frac{1}{2}$  cents per cubic foot, exclusive of the transportation charges and the value of the cylinder, which can be returned to the manufacturer for refilling. The cost of gas for coloring a carload of fruit should not exceed a dollar.

#### EFFECT OF MISCELLANEOUS GASES AND VAPORS UPON THE COLORATION OF GREEN LEMONS

During the course of the experiments, the effect of many substances upon green lemons was tried. A surprisingly large number caused yellowing of the fruit, but in most cases no practical application of the fact could be found, either because of injury to the fruit, expense of the reagent, its poisonous character, etc., or because the color developed was not the bright lemon yellow desired in commercial practice.

Among the substances that induced coloring but caused injury were paracresol, pyridin, ethyl butyrate, methyl amin, bromin, formaldehyde, acetaldehyde, chlorin, amyl nitrite, formic acid, nitric acid, and benzine. Substances causing coloration in strong concentrations but not in low concentrations were acetic acid, hydrochloric acid, trichlorethylene, and amyl acetate. Substances producing fairly good color without injury were gasoline, phenol, and chloral hydrate. Substances producing slight effects or none were calcium hypochlorite, hydroxylamin-hydrochlorid acid, alphanaphthylamin, guaiacol, sulphanilic acid, salicylic acid, benzoic acid, trimethylethylene, kerosene, turpentine, asphaltum, and ethyl ether. Methyl chlorid from a commercial cylinder at a concentration of 1 to 1,000 caused good coloration of lemons in 10 days, and that at a concentration of 1 to 100,000 caused slight coloring. In this case, however, it can not be stated that the methyl chlorid did not contain traces of ethylene.

#### SUMMARY

Some commercially mature lemons are green in color when picked. The desired yellow color must be secured by subsequent treatment.

When forced coloring is desirable, the change from green to yellow is hastened by the use of the combustion gases that arise from kerosene stoves during the burning of kerosene. The object of the investigation here reported was to determine the identity of the gaseous constituent responsible for the coloration of the fruit.

After the mixture of gases from a kerosene stove had bubbled through Pettenkofer tubes filled with bromin water, the residual (unabsorbed) gas failed to induce coloration. This suggested that the effective constituent must be among the unsaturated hydrocarbons.

Ethylene, even in low concentration, caused green lemons to turn yellow. Fruit colored in this way did not differ in any detectable manner

from similar fruit colored in the usual way with kerosene stoves. However, attempts to isolate ethylene from stove gas were unsuccessful.

When mixed with air in varying proportions by volume, concentrations of ethylene down to 1 to 200,000 colored lemons in 5 to 8 days. Concentration down to 1 to 2,000,000 required 6 to 10 days. The lowest concentration tried (1 to 5,000,000) required about 14 days, indicating that this concentration represents the highest dilution at which the rate of coloring is markedly influenced. High concentrations appear to retard coloring, since 80 per cent ethylene for 7 days gave only slight coloring.

Absence of oxygen prevented coloration of the fruit by ethylene.

Coloration was not measurably hastened by ethylene at a temperature of 45° F., but the rate of coloring increased with increasing temperatures from 57° to 82°. A reduction in rate, however, was observed at 93°.

Both ethylene and stove gas increased the rate of respiration of lemons. The carbon dioxide output was increased about 150 to 250 per cent.

Ethylene is now a commercial article and can be bought as a compressed gas in steel cylinders. Gas in measured quantities may be released from the cylinder in a convenient manner and brought in contact with the fruit by use of an apparatus devised for the purpose.

The effect upon green lemons of many other substances, including carbon monoxide, acetylene, methyl chloride, formaldehyde, acetaldehyde, pyridine, amyl acetate, and chlorine, was tested.

#### ACKNOWLEDGMENTS

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# NOCTURNAL PRODUCTION OF CONIDIA BY *SCLEROSPORA GRAMINICOLA*<sup>1</sup>

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## INTRODUCTION

While studying the two conidial *Sclerosporas* (*S. philippinensis* Weston and *S. spontanea* Weston) so destructive to maize in the Philippines, the writer found that in these species conidia are produced only at night when the host surface is covered with dew or other moisture. This naturally raised the question whether *S. graminicola* (Sacc.) Schroet., the type species of the genus, exhibits the same peculiarity. The fact that it does and the bearing of this fact on our knowledge of the importance and relationship of the conidial stage of the species are discussed in the present paper.

The genus *Sclerospora* was established by Schroeter (22)<sup>2</sup> in 1879 on *S. graminicola*, a species which since then has been found quite widely distributed throughout temperate and tropical parts of the world, principally on species of *Setaria*, and rarely on other Gramineae. In the course of its life history *S. graminicola*, like most other *Peronosporales*, passes through two phases of development: The one, characterized by production of immediately germinating conidia, achieving rapid spread; the other, characterized by formation of resistant oospores, serving to insure survival through such unfavorable conditions as winter and drought. In *S. graminicola* the conidial stage, which usually develops first, appearing as a whitish downy growth on the surface of chlorotic areas of the host, generally seems to be of short duration, relatively inconspicuous, rather rare, and involves but little apparent injury to the host. As a result, this stage has not been commonly or abundantly collected and is represented by scanty and unsatisfactory herbarium material. Moreover, it has not been studied in detail. In species of such related genera as *Plasmopara* (10, 28) and *Peronospora* (6, 7, 8), the conidial condition has been investigated intensively, quantitative measurements have been made, restrictions of parasitism have been tested, and morphological aspects of all stages of development have been worked out minutely and illustrated fully. In *S. graminicola*, however, the conidial stage, save in such publications as those of Butler (2), Kulkarni (12), Shirai (24), et al., has been dismissed summarily with brief diagnostic or morphological descriptions, few measurements, and inadequate illustrations.

On the contrary, the oosporic phase of *Sclerospora graminicola* which follows the conidial with a marked distortion and shredding of the leaves and floral parts of the host, is persistent, conspicuous, abundant, and obviously severely destructive to the host. As a result, it has been collected frequently and in abundance, and is well represented in most

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<sup>2</sup> Reference is made by number (italic) to "Literature cited," 783-784.

herbaria; while detailed study has been made on the morphology (2, 12) and cytology (26) of its development, on comparative spore measurements (27), and on the relation to the host attacked (2, 22, 23).

Since the genus was founded, nine other species have been described. Of these, *Sclerospora macrospora* Sacc. (18), which includes *S. kriegiana* Magn., according to Traverso (27), *S. miscanthi* T. Miyake (16), *S. jarlowii* Griffiths (9), and *S. magnusiana* Sorokin (25), have been reported as yet only in the oosporic condition. The remaining five species are alike in that: First, their conidial phase is predominant and destructive, the oosporic being absent or rare; second, their conidia germinate invariably by hyphae; third, they occur in the oriental Tropics and on members of the tribes Maydeae and Andropogoneae. They are thus in contrast to the type species, *S. graminicola*, which is destructive and predominant in its oosporic condition, which typically produces zoospores in conidium germination, and which is of world-wide distribution, mostly on members of the Paniceae.

While investigating two of the conidial *Sclerosporas* of the Orient, namely, *S. philippinensis* and *S. spontanea*, species exceedingly destructive to maize in the Philippine Islands, the writer (29, 30) found that in both, conidia were produced on the leaves of the infected plants only at night when they were covered with a layer of dew. On dewy nights, from about midnight to dawn, the innumerable successively emerging conidiophores formed a conspicuous and luxuriant growth of grayish down, and furnished abundant living material in all stages of development, quite different from the scanty remains, killed and deformed by drying, that persisted for collection or study during the following day. This nocturnal conidiophore production proved to be a very fixed and characteristic process in *S. philippinensis* Weston and *S. spontanea* Weston. Examination of the publications of other investigators suggested strongly that this condition holds also in the other conidial *Sclerosporas* of maize and related crops in the Orient, namely, *S. javanica* Palm (17) of Java, *S. maydis* (Rac.) Butl. (3) of India, and *S. sacchari* T. Miyake (16) of Formosa. Indeed, in the case of the last species, which has been introduced recently into the Philippines (13, 31), there is now no doubt that conidium production is nocturnal, as material collected at intervals during the night and sent to the writer through the cooperation of H. Atherton Lee, of the Bureau of Science in Manila, shows this to be true.

#### PRODUCTION OF CONIDIA IN *SCLEROSPORA GRAMINICOLA*

Whether the type species, *Sclerospora graminicola*, also produces its conidia only at night is a question that naturally presents itself, in view of the fact that this species differs in the several respects already noted from the conidial *Sclerosporas* of the Orient which show this peculiarity. Opportunity to investigate this point occurred while the writer was attending the summer conference of cereal pathologists at the University of Minnesota in 1920. On the grounds of the College of Agriculture, not too far from the plant pathology laboratory which Dean Freeman and Doctor Stakman very generously made available for night work, were found several plants of *Setaria viridis* (L.) Beauv. obviously infected with the conidial stage of *Sclerospora graminicola*.

The fact that the tissue of these plants was pervaded extensively by vigorous mycelium of the parasite, and the leaf surface was marked by

whitish patches of shriveled conidiophores persisting on the leaves from previous productive nights, indicated that the plants were still supporting conidiophore formation under favorable conditions. Frequent careful inspection of these plants throughout the day of July 23, however, showed conclusively that, during this daytime at least, no conidiophore production took place. Accordingly, in the late afternoon, the plants were prepared for night study by removing from the leaves all remnants of previous conidiophore production by carefully scrubbing the surface with moist cotton swabs. That night, beginning at about 8 p. m., when dew deposition commenced, the plants were examined at hourly intervals and were found, indeed, to produce conidiophores in abundance.

By cutting free-hand sections of living, infected leaves, stripping off bits of epidermis, macerating pieces of tissue, and carefully scraping off the down of conidiophores as it developed, the process of conidiophore emergence and conidium production through the night was followed step by step in detail. After production had ceased, as the plants dried off in the early morning of the next day, periodic examination of the plants was continued until afternoon; and this again brought out the fact that no conidiophores were produced during the day. The fact that in *Sclerospora graminicola* conidium formation does indeed occur at night was established by these observations; but in order to supplement them, as they extended over two days and one night only, the most vigorous of the infected *Setaria* plants were transplanted to Washington, D. C., where they were studied further.

The process of conidiophore production involves, as the writer found in the conidial Philippine *Sclerosporas*, the following phases: The preliminary paling of the leaf areas which are to bear conidia, the quantity of conidia produced on such areas, the length of time this production may go on night after night, the importance of moisture in inducing conidiophore production, the development of conidiophores and conidia thus induced, and the nightly schedule followed by this development. The writer did not have opportunity to work out in detail these several points for *S. graminicola* as he did in the Philippine *Sclerosporas*, but studied chiefly the development of the conidiophores and conidia, the nightly schedule followed by this development, and its dependence on dew or similar moisture. The results of this study follow.

#### DEVELOPMENT OF THE CONIDIOPHORES

The conidiophores of *Sclerospora graminicola* develop from the infected *Setaria* leaves only through the stomata, a large proportion of which may be productive. Consequently, conidiophore production is usually more abundant from the under surface of the leaf where stomata are more numerous, although this abundance is dependent also on other factors such as the amount and distribution of dew on the leaf and the length of time successive nocturnal production from the plant has gone on. Beneath stomata from which conidiophores are to emerge, the air chamber is filled with stout, irregularly lobed, densely granular, mycelial branches closely crowded together (Pl. I, A, B), arising from the less conspicuous mycelial strands running between the mesophyll cells. From these substomatal knots, prolongations push through the stomatal slit over which they form a compact group of several minute bulbous out-growths. In some cases there is evidence that the stomatal slit, normally closed at night, is forcibly pushed open by these emerging branches

(Pl. 1, C-F). The bulbous outgrowths next elongate severally to club-shaped stalks which project perpendicularly from the leaf surface and grow rapidly larger (Pl. 1, G, H). At its swollen apex, each of these produces successively the two to four stout primary branches (Pl. 1, I-K); while from these latter similarly arise the secondary branches (Pl. 1, L-O). In like manner, successive series of branches develop until eventually the more or less extensive, usually dichotomous, branch system is complete, and the ultimate tips terminate in tapering sterigmata (Pl. 2, A). The very apex of each sterigma, beginning as a small globular swelling (Pl. 2, B-E), enlarges gradually, until finally it attains the shape and size of the mature conidium (Pl. 2, F), which then is separated from the neck of the sterigma by a wall.

Obviously, the development of the conidiophores and conidia in *Sclerospora graminicola* as just outlined agrees very closely in its several stages with that already established for *S. philippinensis* and *S. spontanea*. Moreover, *S. graminicola* apparently agrees with these Philippine species in the way in which its conidia are shed. While studying the Philippine species, the writer became convinced that the conidia do not fall passively from the sterigmata as has been assumed, but rather are forcibly snapped off when the outbulging of the opposed walls of the basal apiculus of the conidium and of the sterigma tip suddenly overcomes the adhesion of their contiguous surfaces. This point has not been settled for *S. graminicola*; but such indications as the outbulging of the formerly flatly apposed walls of the sterigma tip and conidium base when released, lead to the conclusion that the species resembles those of the Philippines in this respect also.

#### DEPENDENCE ON NOCTURNAL MOISTURE

The process of conidiophore development in *Sclerospora graminicola* adheres to a regular schedule. When the infected leaves were wet with dew, at about 8 p. m., the outgrowths had protruded from the stomatal slit at about 11 p. m., and the first conidiophores and conidia were mature at approximately 2 a. m., while others which had begun to develop meanwhile, matured successively, so that production continued, reaching its greatest abundance at perhaps 3 a. m., and only ceasing when the dew dried from the leaves in the morning sun. This nightly schedule agrees very closely with that found in *S. philippinensis* and *S. spontanea* (32) under conditions of dew deposition normally obtaining in the Philippines.

Conidiophore production in *S. graminicola*, as in the Philippine species, is vitally dependent on the presence and persistence of dew or other moisture on the leaves. Infected *Setaria* plants that were kept dry during the night never showed conidiophore formation even though their leaves were obviously thoroughly invaded by vigorous mycelium, while similar plants exposed to dew supported abundant conidiophore production. After the leaf surface of infected plants had been wet with dew for about five hours, production began; if the moisture dried off prematurely, production coincidentally ceased; if moisture persisted unduly into the morning, production was thus much prolonged. As in the Philippine *Sclerosporas* also, conidium production in *S. graminicola* is very sensitive in its response to moisture changes, so much so that, when studied at the University of Minnesota, the *Setaria* plants in such different localities as in a glade among trees, at the edge of a wood on a small hill, and in an open lot, showed slight variations in their schedule of

conidiophore production as a result of local differences in amount or time of dew deposition.

How long *Sclerospora graminicola* may continue nocturnally producing conidia on infected *Setaria* plants, how tolerant of this continued production the host may be, and how much of its life span may be taken up by the conidium-forming period of the fungus, are points which, in this case, were not worked out in detail as they had been for the Philippine *Sclerosporas* (32). In one case, however, even though the transplanted *Setarias* grew very poorly, one plant showed intermittent conidiophore formation for more than two weeks. Also, in the field, there is abundant evidence that the period of conidium production continues much longer than this, even extending over as great a proportion of the total life of the host as did the *Sclerosporas* studied by the writer (32) on maize in the Philippines.

A study of large numbers of *S. graminicola*-infected *Setaria* plants of various ages in the fields near the College of Agriculture at St. Paul, Minn., showed the progress of the disease to be as follows: Production of conidia begins on newly unfolding leaves in symptomatically chlorotic streaks which, by their extent and position even in very young plants, indicate a fundamental systemic infection starting in the young seedling and giving rise to thorough and extensive invasion of the host tissue. The production of conidia continues during each favorable night on these earlier leaves, and also continually starts up afresh on leaves successively appearing. Gradually, as the host matures, conidiophore formation from the host surface is superseded by the development of oogonia on the intramatrical mycelium, beginning in the lower, first unfolded, older leaves, and working slowly upward. The uppermost, latest, and youngest leaves are the last to be affected, and may continue abundant conidiophore production until the head, usually deformed, sterile, and virescent, is full grown. Finally, however, even these ultimate leaves also are given over to oogonium formation, and show the shredding of tissue which marks the maturity of the oogonial phase of the fungus.

The conidial stage of *Sclerospora graminicola*, because its relation to nocturnal moisture has not been understood, has been considered from the time of Schroeter to the present as fleeting, transitory, fugacious, and evanescent. Now, however, that we know the period of conidium production may be of relatively long duration, we must alter our conception of the evanescence of the conidial stage. It is fugacious in the sense that conidiophore production takes place for only a few hours during the night; but it is decidedly persistent in the sense that this production may go on night after night throughout a large proportion of the total life of the host.

#### CONIDIAL SCLEROSPORA GRAMINICOLA COMPARED TO OTHER SPECIES

Material of the conidiophores and conidia of *Sclerospora graminicola* gathered thus at night during the period of optimum conidiophore production is infinitely more satisfactory for study than the dried and shriveled remnants which persist from previous nights and may be collected during the day. Consequently, such material gives a somewhat broader conception of the character of the species than that which we gain from the usual descriptions and figures based on dried material, and also permits a more adequate comparison with the conidiophores and conidia of other species.

## CONIDIOPHORES

In the case of the conidiophores, for example, a study of optimum nocturnal material shows clearly that the conidiophores are larger, more extensively branched, and structurally more complex and elaborate, than most of the illustrations and descriptions indicate. The first description of the conidial stage of this species was published by Schroeter (22) and the first illustrations were shown by Fischer (5, fig. 71). Together these present a vivid characterization of the conidiophores as decidedly short (about  $100\mu$ ) and thick (about  $12\mu$ ) with few stubby branches bearing a relatively small number of conidia (about 15). No appreciable departure from this characterization is found in the publications by such subsequent investigators in Europe as Berlese (1), Malbranche and Letendre (14), Massee (15, pl. 1, fig. 16), Saccardo (19), et al.; or such in America as Saunders (21, pl. 16, fig. 4), Farlow (4), Wilson (33), et al.; or in India as Butler (2, fig. 6, 7), and Kulkarni (12, fig. 1-3). In Japan, however, Shirai (24, fig. 16, 17) described the conidiophores as much larger ( $100$  to  $240\mu$  by  $12$  to  $19\mu$ ) and with a somewhat more elaborate branching system; but the fact that he found conidia of two strangely different size-classes ( $24$  to  $28.8\mu$  by  $16.8$  to  $19.2\mu$ , and  $38.4$  to  $57.6\mu$  by  $19.2$  to  $24\mu$ ) arouses the suspicion that he was not dealing with *S. graminicola* alone, and to some extent invalidates his characterization of the species.

The writer, after studying the conidiophores of *Sclerospora graminicola* in the progressive stages of their development under optimum conditions at night, is convinced that the descriptions and drawings of the investigators just mentioned are based on nontypical, poorly developed specimens, the last belated stragglers of the nocturnal production, caught by the morning sun before they had developed conidia, and dried to a condition still less typical and favorable for study. In contrast to such dried specimens, material scraped from *Setaria* leaves at the time of optimum nocturnal production shows conidiophores that are much larger and better developed than we had been led to believe characteristic of *S. graminicola*, and which approximate, in this respect, those of such luxuriant species as the conidial *Sclerosporas* of the Orient. If, for example, one compares the accompanying figures of *S. graminicola* (Pl. 2, I, K, O) with those of *S. spontanea* (30, pl. 79, fig. A), and *S. philippinensis* (29, pl. 24, fig. O, pl. 25, fig. B) all of which are of very nearly the same magnification, it is obvious that they show a general resemblance which is not even suggested by previous illustrations.

After comparing them carefully, however, the writer finds that even the largest and most elaborate conidiophores of *S. graminicola* differ markedly in certain essential features from the conidiophores of such oriental species as *S. philippinensis* and *S. spontanea*.

First, the total length in these Philippine species is as a rule much greater ( $260$  to  $400\mu$ ) than in the case of *S. graminicola*, even the most luxuriant nocturnal material of which has a length of about  $150\mu$  with occasional extremes as low as  $100\mu$  or as high as  $200\mu$ .

Second, the branching system also shows differences which are much more qualitative and absolute than are mere distinctions in size. In the Philippine species, for example, usually three, sometimes two or four, primary branches of approximately equal size and extent of development all spread out at angles of about  $45^\circ$  or less from the main axis in very close succession; and all are of equal rank—no one of them being either in direction, position, or extent of growth more to be considered a continua-

tion of the main axis than any other. As a result of this also, the conidia arising from the branch tips are arranged approximately in a hollow hemisphere. In *S. graminicola*, on the contrary, one of the primary branches stands out more or less obviously as a continuation of the main axis (Pl. 2, I, K, L, O, Q) both in direction and in extent of growth. From this continuation of the main axis other main and secondary branches grow out at irregular intervals, usually at angles of  $45^{\circ}$  to  $90^{\circ}$ . As a result, the conidia at the ends of the branches lie more frequently in irregularly disposed groups (Pl. 2, I, K, L, P) than in an approximate hemisphere (Pl. 2, O, Q).

Third, the sterigmata of *S. graminicola*, as a rule, are shorter and more broadly bottle or tenpin shaped (Pl. 2, N, T, V, W) than are those of the Philippine *Sclerosporas*. The shape, however, is somewhat dependent on position, single sterigmata at the tips of the branches (Pl. 2, U) being more elongate than those borne in groups of two, three, or four (Pl. 2, T, J, W). Moreover, the sterigmata, which in the Philippine *Sclerosporas* almost invariably continue the direction of the branch tip that bears them, may stand out from the branch tip even at right angles in *S. graminicola* (Pl. 2, I, J, L). Finally, the lower part of the conidiophore of *S. graminicola* is not usually marked off by a cross wall into a basal cell or foot cell that is distinct from the superior portion of the main axis. Occasionally such basal cells are encountered (Pl. 2, K, X, Y); but this condition is the exception; and hence is in distinct contrast to the Philippine species in which it is a characteristic feature. Occasionally, also, the base is distinguished by an incomplete transverse septum (Pl. 2, K) or by a decided thickening of its longitudinal wall (Pl. 2, A, P, Z).

All these distinctions—size of the conidiophores, character and extent of the branch system, form and direction of the sterigmata, and extent of septation or thickening at the base of the main axis—are matters of degree which should be expressed quantitatively to facilitate comparisons. Yet, even when considered as qualitative differences, they show clearly that the conidiophores of *S. graminicola*, though they may approximate those of such typically conidial oriental forms as *S. philippinensis* and *S. spontanea* in luxuriance and general appearance, are indeed distinct from them.

#### CONIDIA

It is the conidia themselves, however, that are the most distinguishing feature of *Sclerospora graminicola*. These bodies differ markedly from those of other *Sclerosporas* in size, shape, structure, and germination. The size of the conidia varies, and for adequate presentation requires quantitative expression based on measurements of large numbers of conidia. Moreover, to be ideally satisfactory the conidia should be caught on the slide in dew when snapped off from the conidiophores at maturity and measured at once. Unfortunately, the writer was not able in this case to make all the measurements under these ideal conditions as he did for the Philippine *Sclerosporas*. Rather, most of the measurements were made from material scraped from abundantly productive leaves at 3 a. m., then killed by Flemming's weaker solution, and mounted in dilute glycerin and eosin. A comparison of measurements made thus with the relatively few which the writer had opportunity to make from fresh material indicates that if all the measurements had been made under ideal conditions the modes of length and of diameter probably would have been

increased by one  $2\mu$  class (from 18 to  $20\mu$  and from 14 to  $16\mu$ ). With this probable correction in mind, however, the 400 measurements<sup>3</sup> here pre-

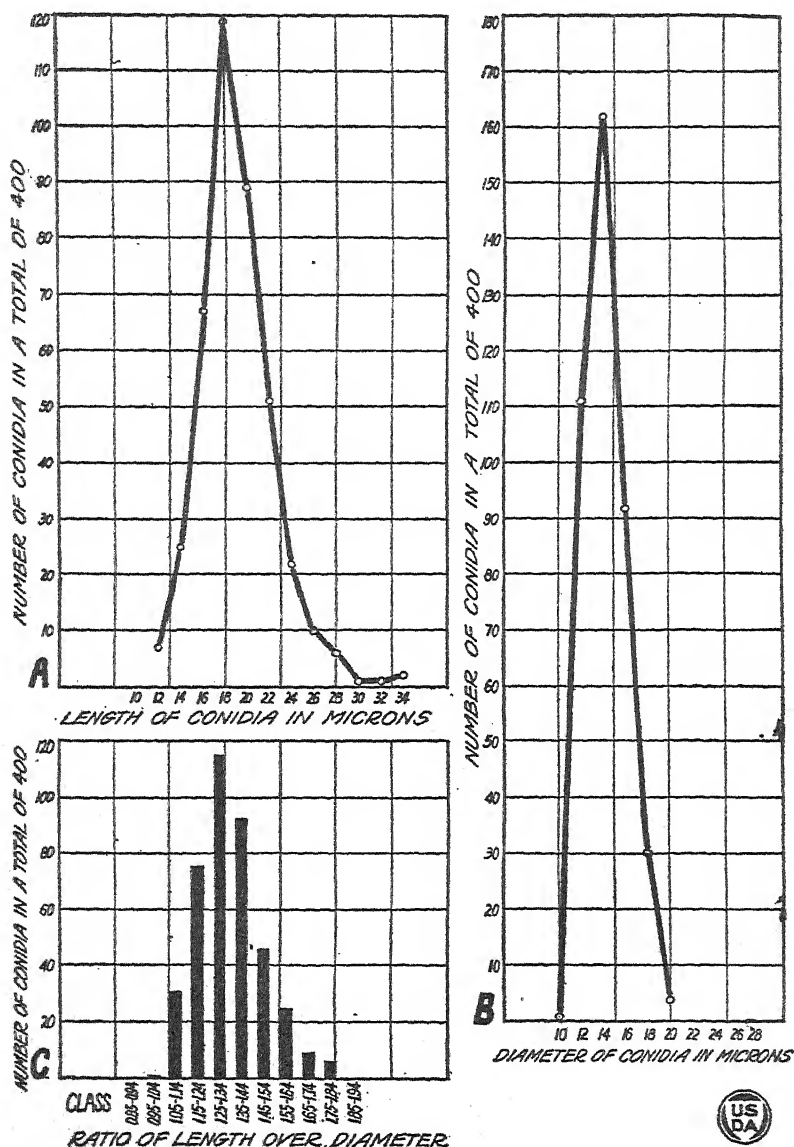


FIG. 1.—Diagrams showing the frequency of occurrence of various sizes encountered in 400 conidia (20-*osporangia*) of *Sclerospora graminicola*; A, variation of the conidia in length; B, variation of the conidia in diameter; C, ratios of length to diameter.

sented (fig. 1 and Table I) offer a fair basis of comparison with other species.

<sup>3</sup> The writer is indebted to Miss Margaret Kemp, graduate student in botany at Radcliffe College, for making one half of the measurements embodied in the table and graphs.



TABLE I.—Measurements, and ratios of length to diameter of 400 conidia (zoosporangia) of *Sclerospora graminicola* arranged in size and ratio classes

Length.		Diameter.		Ratio, length to diameter.	
Classes.	Number of conidia in 400.	Classes.	Number of conidia in 400.	Ratio classes.	Number of conidia in 400.
$\mu$		$\mu$			
11 to 12.9	7	9 to 10.9	1	0.95 to 1.04	1
13 to 14.9	25	11 to 12.9	111	1.05 to 1.14	31
15 to 16.9	67	13 to 14.9	162	1.15 to 1.24	75
17 to 18.9	119	15 to 16.9	92	1.25 to 1.34	115
19 to 20.9	89	17 to 18.9	30	1.35 to 1.44	92
21 to 22.9	51	19 to 20.9	4	1.45 to 1.54	46
23 to 24.9	22			1.55 to 1.64	25
25 to 26.9	10			1.65 to 1.74	9
27 to 28.9	6			1.75 to 1.84	6
29 to 30.9	1				
31 to 32.9	1				
33 to 34.9	2				

From the measurements presented above in graphic and tabular form, it is obvious that, although conidia are encountered which measure from 12 to  $34\mu$  in length and 10 to  $20\mu$  in diameter, the greater proportion of them are between 16 and  $22\mu$  long by 12 to  $16\mu$  in diameter. These measurements present an interesting comparison to those previously recorded for *S. graminicola*. In Europe, Schroeter (23, p. 115) gave the size of the conidia as  $20\mu$  long by 15 to  $18\mu$  wide, while Saccardo (19), apparently using scanty and immature material, first recorded 12 to  $15\mu$  by 10 to  $11\mu$ , and later (20, p. 238) modified this to 12 to  $20\mu$  by 10 to  $18\mu$  to include Schroeter's figures. Others, such as Malbranche and Letendre (14), Fischer (5, p. 437), and Berlese (1, v. 9, p. 70) followed these two without making original measurements themselves. In the United States, Saunders (21, p. 58) is apparently the only one to give original measurements, 18 to  $20\mu$  by 13 to  $15\mu$ , the others, from Farlow (4) to Wilson (33), following Schroeter and Saccardo. In the Orient, Butler (2) gave 22 to  $30\mu$  by 12 to  $16\mu$  and Kulkarni (12) 19 to  $31\mu$  by 16 to  $21\mu$  for *S. graminicola* in India; while Shirai (24) for Japan, gave 24 to  $28.8\mu$  by 16.8 to  $19.2\mu$  for the usual size, but made the startling statement that at times giant conidia,  $38.5$  to  $57.6\mu$  by  $19.2$  to  $24\mu$ , occurred. Although no exact comparison can be made with inclusive, limiting, nonquantitative measurements such as these, there seems to be a fair agreement between the European and United States figures and those of the writer. The measurements from the Orient apparently show a consistently greater length; but whether this indicates different specialized races or merely greater luxuriance of growth under tropical conditions remains to be determined. The remarkable discrepancy in size and shape reported by Shirai arouses the suspicion that he was dealing with two species, and demands thorough corroboration before this larger size is included as a correct measurement for *S. graminicola*.

In shape, the conidia vary considerably, ranging from subspherical through ovoid and obovoid to ellipsoid, lemon-shaped, and rounded-

cylindric. Fully mature spores are perhaps most commonly broadly ellipsoid or broadly cylindric. A qualitative idea of the usual variations in form may be gained from the representative conidia grouped in Plate 2, M. A quantitative idea of the relative predominance of the short, broad shape is given by the ratios of length to diameter which are grouped in Table I (p. 779) and in the diagram (fig. 1).

Obviously, both in their small size and rotund shape the conidia, as revealed in this nocturnally collected material, mark *Sclerospora graminicola* as distinct from the other conidial Sclerosporas—namely, the typically ellipsoid, large-spored species of the Orient. *Sclerospora javanica* of Java, however, if we may judge from the 20 measurements given by Palm (17), has rotund conidia ranging from 22 to 26 $\mu$  in length by 16 to 20 $\mu$  in diameter, but most commonly 24 by 18 $\mu$ , a size so closely approaching that of the conidia of *S. graminicola* reported from Japan by Shirai (24) and from India by Butler (2) and Kulkarni (12) that distinction based on conidia alone might be difficult were it not for the considerably different structure and germination of these spores.

The wall of the conidium is thin (0.5–1 $\mu$ ) and of cellulose save at the apex where, as the conidium matures, the single so-called papilla of dehiscence develops (Pl. 2, G, M, O, T). This is a specialized tip, approximately plano-convex in shape, although of variable thickness and area, and of modified cellulose (pectin or hemicellulose) composition. At the opposite (basal) end of the conidium there persists in some cases an apiculus of attachment (Pl. 2, G), which is merely the point at which the conidium was affixed to the sterigma and is not at all comparable to the apical papilla.

At germination the apical papilla of dehiscence softens and gelatinizes, leaving a terminal pore (Pl. 2, H) through which escape the several zoospores into which the granular conidium content has by then become differentiated. The conidium is thus in effect a zoosporangium, but like many other Phycomycete zoosporangia it has the potentialities of a conidium and may, under circumstances unfavorable to zoospore emergence, germinate by sending out hyphae. Under ordinary circumstances, however, hyphal germination is rare, the writer having seen only a few instances among many observations. Also, even when germinating by hyphae, the conidia are distinguished by the presence of the apical papilla of dehiscence from conidia of other species of *Sclerospora* in which hyphal germination is the rule.

The process of germination, involving as it does the maturing of the zoosporangium, the development of the zoospores, the formation of the apical papilla of dehiscence, the deliquescence of this structure, and the escape and subsequent behavior of the zoospores, shows many points of interest which the writer hopes to work out in detail and present at a later date.

It is the conidia, then, as they appear in favorable night-collected material which particularly distinguish the conidial stage of *Sclerospora graminicola*. Their short, thick form and small size, although relative characters, are of some diagnostic value in distinguishing the species. The possession of an apical papilla of dehiscence, however, is the one absolute distinction. By this character, and secondarily by the zoosporic germination which usually follows, *S. graminicola*, as yet, must stand as a unique representative of the genus. In this connection, Kulkarni's (12) study on this species in India is of especial interest. This investigator found that, although the characteristic oogonial stage of *S. graminicola*

on *Pennisetum typhoideum* Rich. and *Andropogon sorghum* (L.) Brot. is apparently the same on both hosts, the conidial stage found on the latter host differed markedly from the typical conidial *S. graminicola* which developed on *Pennisetum*. Conidia produced on *A. sorghum*, by their distinctly subspherical shape, absolute lack of an apical papilla of dehiscence, and invariable germination by tubes, consistently present, even though agreeing in size, a very decided contrast to the broadly elliptic, apically papillate, zoospore-forming conidia of the type. Moreover, the branch system of the *Andropogon* fungus was more extensive than that of the type, and the sterigmata reached a length of  $16.3\mu$  while those of the type were but  $8.3\mu$ . Also, the fungi showed differences in their effect on the two hosts in the field and in their failure to produce cross infection. As a result, Kulkarni establishes the fungus on *A. sorghum* as *S. graminicola* var. *Andropogonis sorghi*.

In the opinion of the writer, this is certainly a distinct species, one which, aside from other differences, needs only the absolute criterion that its conidia lack an apical papilla of dehiscence to distinguish it without question from *S. graminicola*. It is clearly a species closely allied to the destructive, predominantly conidial *Sclerosporas* of the Orient even though it is apparently connected with an oogonial stage, presumably that of *S. graminicola*. Also, it promises most interesting results if studied intensively through numerous cross inoculations, comparative measurements of large numbers of conidia, and persistent efforts to determine whether it is or is not actually genetically connected with the typical *S. graminicola* oogonia on various hosts. Even before such an investigation is made, however, we are, in the writer's opinion, justified in regarding the apical papilla of dehiscence of the conidia of *S. graminicola* as a diagnostic feature of absolute value—a feature as yet confined to this species alone.

#### GENERAL DISCUSSION

Because it differs, in the respects which have been discussed, from all other known conidial members of the genus, there is the more significance and interest in the fact that *Sclerospora graminicola* shows the same main features of nocturnal conidiophore production which characterize at least three of these other conidial species. This fundamental agreement in behavior, together with the general similarity in structure and development, is sufficient, in the opinion of the writer, to outweigh the difference in germination. It seems undesirable, therefore, to establish a new genus on the species whose conidia germinate by hyphae, at least until much more extensive comparative study of the several species has given us further basis for such a rearrangement. Within the genus, however, there might be advantages in following Ito's (11) suggestion of establishing one subgenus, *Eusclerospora*, to include *S. graminicola*, and another, *Peronosclerospora*, to comprise the species the conidia of which germinate directly.

Of the *Sclerosporas* with known conidial stages, four species, *S. philippincensis*, *S. spontanea*, *S. sacchari*, and *S. graminicola*, alike have been found by the writer to be characterized by nocturnal conidiophore production. It will rest with future investigation to justify the natural assumption that this feature is common to all conidial *Sclerosporas*. It will rest with future investigation also to decide with finality what factor or combination of factors is operative at night to induce conidiophore

production. These observations on *S. graminicola* corroborate the writer's earlier decision (32), that persistent dew or other moisture on the infected leaves is of primary importance. More precise physiologic study probably will show, however, that the relationship is more subtle and complex than this would imply.

In any case, now that we know that *Sclerospora graminicola* produces its conidia only at night when the infected leaves are covered with a layer of moisture, we are in a position to study the dispersal of the conidia, their relation to the dissemination of the species, and the part the conidial stage as a whole plays in the life history of the fungus; to investigate intensively the physiologic and morphologic aspects of its parasitism, and the immunity or susceptibility shown by more or less related hosts; in short, to determine facts of immediate application toward the control of this destructive parasite.

#### SUMMARY

In the peronosporaceous genus *Sclerospora*, oogonial and conidial stages are known. Both of these develop regularly in the type species, *S. graminicola* (Sacc.) Schroet. Of the nine remaining species four are known as yet only in the oosporic condition. All the other five alike are predominant and destructive in their conidial phase—the oogonial being absent or very rare; all show germination of the conidia by hyphae; and all occur in the oriental Tropics on grasses of the tribes Maydeae and Andropogoneae.

To these five conidial species, *S. graminicola* is apparently in distinct contrast; for, although it does, indeed, develop a conidial stage, the oogonial condition is the predominant and obviously destructive one; the conidia (zoosporangia) germinate by zoospores; and the distribution is world wide—the host plant usually being a grass of the tribe Paniceae (Setaria, etc.).

Despite these differences, however, the writer finds that the conidial phase of *Sclerospora graminicola* shows certain of the same fundamental features of development which he has found recently to be always present in three of the five typically oriental species. The first fundamental feature is that *S. graminicola* produces its conidiophores only at night and when the surface of the infected leaves is covered with a layer of dew or similar moisture.

The second fundamental feature is that this production of conidiophores runs a well-defined course as follows: The knots of stout hyphae crowded in the substomatal air chambers push out prolongations through the stomatal slit, and form bulbous outgrowths which elongate successively to clavate stalks; and these in turn develop at their tips a more or less extensive branch system, and ultimately sterigmata and conidia.

The third fundamental feature is that this process of conidiophore production follows a regular nocturnal schedule which is vitally dependent on the presence and persistence of dew or other moisture on the leaf surface.

The writer describes and illustrates conidiophore development in *S. graminicola* and compares the species with *S. philippinensis* and *S. spontanea* in the regularity and dependence on moisture of its nocturnal schedule. The fact that conidiophore production is nocturnal only, and that during the day there remain only remnants of the previous night's

crop, and that the spores and conidiophores can not survive desiccation explains why so little concerning the conidial stage has been known hitherto.

When studied in a fresh condition as they form at night, the conidiophores are found to be larger and much more elaborate and complex than one would have assumed from previous descriptions and illustrations—even approximating in luxuriance the conidiophores of the typically conidial species of the Orient. Nevertheless, *S. graminicola* stands as distinct from all other species now known. Its conidiophores have certain essentially characteristic features; while its conidia each develop an apical papilla of dehiscence, and hence germinate by emitting zoospores.

The fugacity of the conidial stage has been overemphasized heretofore; for, although it is fugacious in the sense that conidiophores develop only for a few hours each night, yet it is distinctly persistent in that this development may be repeated night after night for a considerable part of the life of the host.

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# PLATE I<sup>o</sup>

A.—Mycelium of *Sclerospora graminicola* within a portion of a cross section of a badly infected *Setaria viridis* leaf cut at 10 p. m. The invading hyphae have pushed the mesophyll cells apart, and knobby branches have made their way into the air chamber under the stoma through which they will grow out and give rise to conidiophores. At *a*, a small, bulbous haustorium has penetrated an epidermal cell, and at *b* a branch of the mycelium appears in cross section as it runs between the mesophyll cells at right angles to the section.  $\times 425$ .

B.—A bit of *Setaria* leaf like that shown in A, but more highly magnified, and cut in longitudinal section somewhat later at night. The mycelium has invaded the tissue more extensively, and has developed in the air chamber a crowded group of proliferating branches, some of which are just about to push out through the stomatal slit.  $\times 850$ .

C.—Exterior view of a stoma in a piece of epidermis cut from an infected leaf of *Setaria viridis* at midnight. A knoblike protrusion has grown out from the proliferating branches, which are shown crowded in the substomatal air chamber in B, and is pushing through the partly closed stomatal slit ready to elongate into a conidiophore initial. 12 p. m.  $\times 375$ .

D-E.—Early stages in the development of the conidiophores of *Sclerospora graminicola* from the leaves of *Setaria viridis*, showing the elongation of the knoblike outgrowths, seen in C, into club-shaped conidiophore initials. 12 p. m.  $\times 375$ .

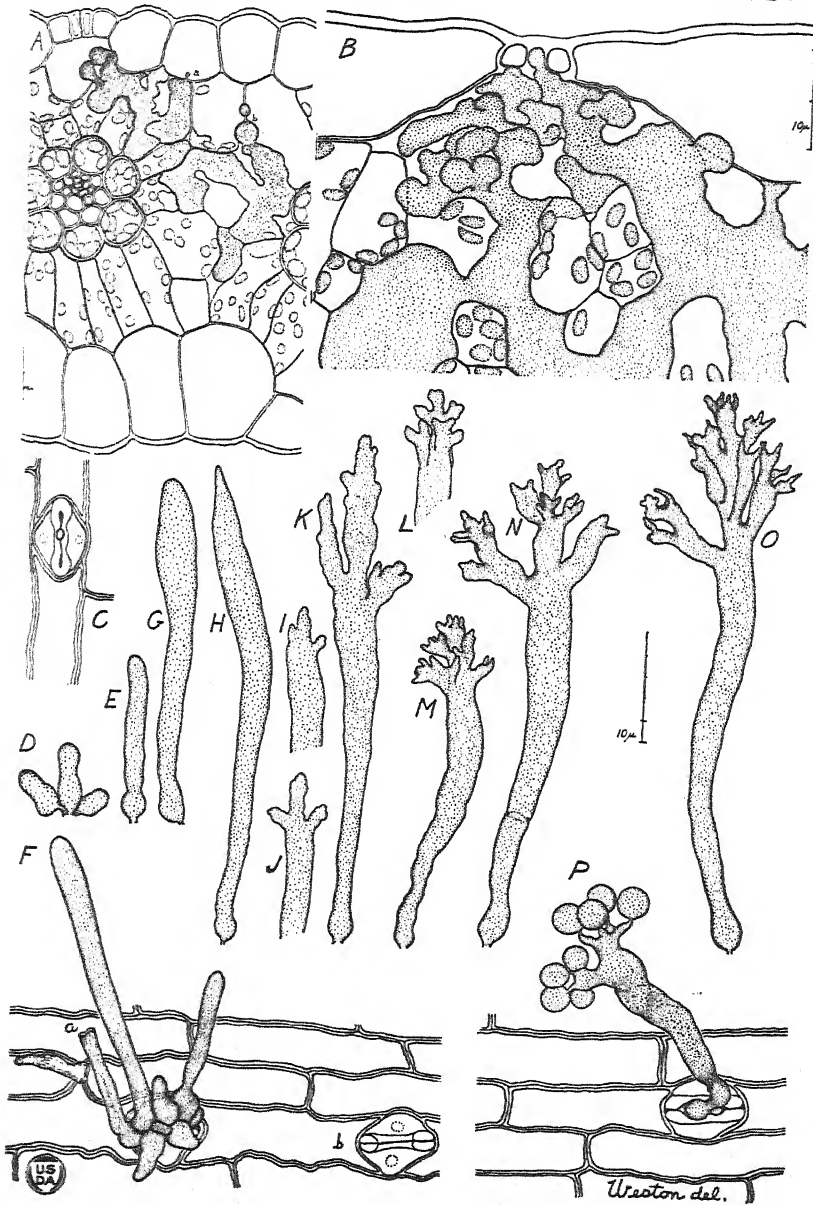
F.—Exterior view of a bit of the epidermis of a *Setaria* leaf, looking obliquely at a clump of young conidiophore initials arising from the almost obscured stoma, and showing various stages of development, from young knoblike outgrowths that have just emerged to older elongate stalks. At *a* is shown the base of a conidiophore already matured, and now shriveled and broken off; and, at *b*, a stoma as yet uninvaded by the fungus. 12 p. m.  $\times 375$ .

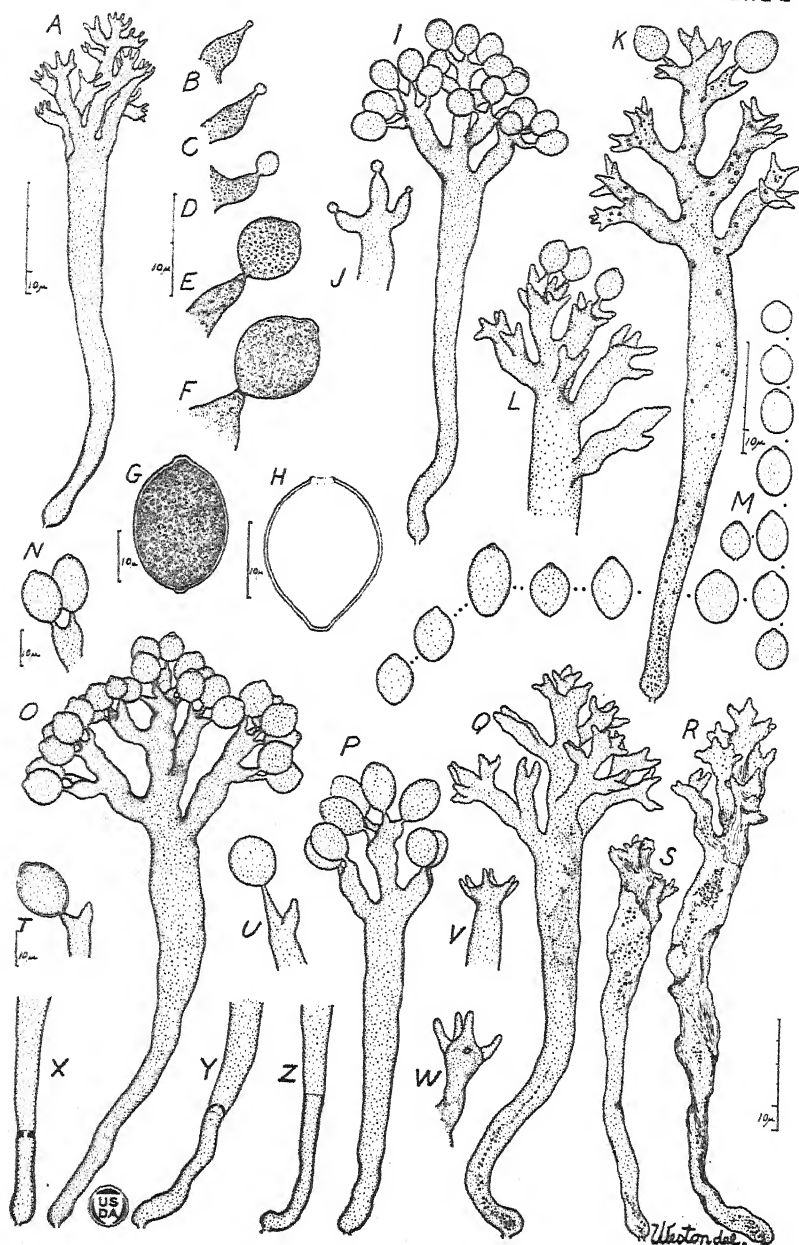
G-O.—Later stages in the development of the conidiophores, showing the formation of the successive series of branches (primary, secondary, etc.), until finally at O the branch system is complete. 3 to 3 a. m.  $\times 375$ .

P.—A bit of the upper epidermis cut from an infected *Setaria* leaf at 3 a. m., and showing, in oblique view, a maturing conidiophore. This conidiophore is an unusually stunted one, bearing only eight still spherical and not yet mature conidia, on a reduced branch system of two primary branches each of which gives rise directly to four sterigmata and conidia. 3 a. m.  $\times 375$ .

<sup>a</sup> The drawings were made with the aid of a camera lucida, and are all of *Sclerospora graminicola* on *Setaria viridis*. A, B, C, F, and P are drawn from free-hand sections of living material cut at night in water, and immediately killed and fixed in Flemming's weaker solution, stained, and mounted. D, E, and G to O are from material carefully scraped from productive leaves at night, and either drawn at once while still living, or drawn after being killed and mounted as were the sections. The approximate magnification of the printed figure after its reduction from the original drawing is given in each case, as is also scale with 100 divisions as an absolute measure.







A.—A conidiophore of *Sclerospora graminicola* with the branch system completed and its ultimate tips elongating into sterigmata which will give rise to the conidia. The wall at the base of the main axis is thickened into a differentiated, footlike portion.  $\times 375$ .

B-F.—Successive stages in the development of the conidia from the tips of the sterigmata. Note that at a relatively early stage, the wall at the tip begins to thicken and becomes modified into the characteristic apical papilla of dehiscence.  $\times 850$ .

G.—Mature, recently shed, conidium with well-developed papilla of dehiscence at its apex, and the small apiculus by which it was attached to the sterigma still persistent at the base. The content has not yet begun to divide into zoospores, but is still undifferentiated and granular.  $\times 850$ .

H.—The empty wall of a conidium (i. e., zoosporangium) which has germinated by emitting zoospores through the pore left by the softening and dissolution of the terminal papilla of dehiscence.  $\times 1,200$ .

I.—Conidiophore at a later stage than that shown in A, with a vigorous, well-developed branch system which bears 18 only partly developed conidia. These conidia are still spherical, and have not as yet developed the terminal papillae of dehiscence.  $\times 375$ .

J.—Detail of a branch tip of the three-pronged type frequently encountered (cf. fig. 1). Note that the young conidia which are just beginning to develop from the three sterigmata are not of exactly the same stage of development.  $\times 600$ .

K.—A large, well-developed conidiophore which has shed all but two of its conidia. The branch system shows a somewhat extreme case of the continuing of the main axis, a characteristic of *Sclerospora graminicola*. An attempt has been made to show the detail of the content as it appears when the conidiophore is at this stage. An incomplete septation at the base delimits the basal cell which is occasionally encountered in this species.  $\times 375$ .

L.—A still more extreme case of continuation of the main axis in the branch system of the conidiophore.  $\times 375$ .

M.—Representative conidia showing the various shapes and sizes most frequently encountered. Note that each has a terminal papilla of dehiscence. The content, which is conventionally stippled here, is shown in detail in G.  $\times 375$ .

N.—Detail view of two conidia still in place but already mature and about to be shed from the sterigmata.  $\times 600$ .

O.—Typical, vigorous, well-developed conidiophore, like that in figure 1, but in a later stage of development. This is shown by the maturity of its 24 conidia, their larger size, more rotund ellipsoidal shape, and prominent papillae of dehiscence. Note the absence of any foot cell or basal thickening of the main axis wall. 2 a. m.  $\times 375$ .

P.—A small, stunted conidiophore. The branch system, in comparison to that of a typical well-developed individual such as the one shown in O, is much reduced. Its three primary branches give rise directly to eight sterigmata and conidia, which, however, are larger than the more numerous ones of O. Note that the wall of the main axis is thickened at its base. 3 a. m.  $\times 375$ .

Q.—A typical, well-developed conidiophore which only recently has shed its 34 conidia, as is shown by the bottle-shaped and rounded tips of its sterigmata, and by the beginning of the disintegration of its content. 3 a. m.  $\times 375$ .

R.—The shriveled and shrunken remains of such a conidiophore, which had developed during some previous night, and had remained, dried, on the leaf until scraped off and examined during the day.  $\times 375$ .

S.—A similar, mummified conidiophore, but one more completely shrunken and collapsed after longer drying.  $\times 375$ .

T.—Detail of a branch tip of the dichotomous type so frequently encountered. One sterigma still bears a mature conidium; while the other by its bulging, rounded apex shows that it has only recently discharged.

U.—Detail of a similar branch tip with one typical, short sterigma which has already shed its conidium, and one rather unusually elongate sterigma bearing a conidium only partly developed.  $\times 600$ .

V.—Detail of a branch tip with six sterigmata which have a somewhat uncommon arrangement resembling slightly that of the sterigmata of *Bremia*.  $\times 600$ .

W.—A branch tip of a type frequently encountered, showing four approximately equal pronglike sterigmata standing out at equal distances from the club-shaped branch tip.  $\times 600$ .

X-Y.—Typical examples of the kind of basal cells occasionally found in conidiophores of *Sclerospora graminicola*.  $\times 375$ .

Z.—Base of a conidiophore showing the thickened wall that at times marks off a differentiated foot portion instead of a basal cell.  $\times 375$ .

\* The drawings were made with the aid of a camera lucida, and are all of *Sclerospora graminicola* on *Scleria tridris*. Figures H and I were drawn from living material; the others from material scraped from leaves bearing abundant conidiophores at the time of maximum nocturnal production, and immediately killed with Flemming's weaker solution, stained (chiefly with safranin).



# DAILY VARIATION OF THE CARBOHYDRATES IN THE LEAVES OF CORN AND THE SORGHUMS<sup>1</sup>

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## INTRODUCTION

In a comparative study of the physiological functions of corn (*Zea mays*) and the sorghums (*Andropogum sorghum*), it was thought advisable to make some observations on the variations of the carbohydrates in the leaves of these plants during a 24-hour period. It was considered that any data obtained in a study of this kind would be of value not only in interpreting the different behavior of these two types of plants when grown under severe climatic conditions, but also in helping to gain a better general knowledge of the fundamental physiological processes of agricultural plants. The observations reported in this paper were made upon plants growing at Garden City, Kans., in 1916 and 1917, and at Manhattan, Kans., in 1919.

## HISTORICAL

The basis of our knowledge of the carbohydrates of the leaves was laid by Sachs (12, 13, 14)<sup>3</sup> when he proved that the appearance of starch in the chloroplasts is a direct outcome of the fixation of carbon under the influence of sunlight and chlorophyll. He further stated that starch is the first visible product of carbon assimilation and that it is translocated from the leaves in the form of sugar. Kayser (8) found both cane sugar and reducing sugar in the leaves of the beet, grape, potato, and onion and succeeded in separating cane sugar in the crystalline form from the leaves of the grape. Girard (7) found that the amount of cane sugar in the leaves of the beet increased during the day, but that the amount of hexoses remained approximately constant. Schimper (15) concluded that glucose formation precedes starch formation in the leaves and that starch is formed from glucose when its concentration exceeds a certain maximum, which differs in different plants. Meyer (9) observed that certain plants form little or no starch in their leaves and that when starch is found in leaves the amount of sugar present is relatively low, while when starch is absent the sugar content is relatively high. Brown and Morris (1) in their classical work on the carbohydrates of the leaves of nasturtium considered that the dextrose and laevulose present in the leaves are more readily accounted for as the products of the hydrolysis of cane sugar than as its precursors. On account of the relative amount of cane sugar found in the leaves and the manner in which it

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<sup>2</sup> The carbohydrate determinations were made by the Department of Chemistry, Kansas Agricultural Experiment Station, the funds for the work being furnished by the Department of Botany. Acknowledgments are due Prof. W. L. Latshaw, under whose supervision the chemical analyses were conducted.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 807-808.

fluctuates during the day, they concluded that it is the first sugar formed in photosynthesis. Strakosch (17) studied the sugars in the leaves of the sugar beet by microchemical methods and concluded that dextrose is the first sugar formed in the mesophyll of the leaves of this plant. Parkin (11) from his observations on the carbohydrates of the leaves of the snowdrop (*Galanthus nivalis*) expressed the opinion that cane sugar is the first recognizable sugar to appear in the leaves of this plant and that the dextrose and laevulose rise from the inversion of cane sugar. He also found that there is more cane sugar in proportion to reducing sugars in the leaves during the earlier part of the growing season than during the latter part. Davis (3, 4, 5) and his coworkers concluded from their investigations of the carbohydrates of the leaves of the mangold and potato that cane sugar is the first sugar formed in the mesophyll of the leaves under the influence of chlorophyll and sunlight. They believed that this cane sugar is transformed into the hexoses in the veins and midribs of the leaves and that it is translocated to the places of storage in that form. They observed, as did Parkin, that in the earlier part of the season cane sugar is present in the leaf tissue in excess of the hexoses, but later in the season the reverse is true. Dixon and Mason (6) concluded from microchemical tests that hexoses are the first sugars formed in photosynthesis in the chloroplasts and that the comparatively large amount of cane sugar observed in chemical analyses is only a temporary storage product in the vacuoles of the green cells. Spoehr (16) in his observations on the seasonal variation of carbohydrates in *Opuntia phaeacantha* found that a low water content and high temperatures are associated with an increase of polysaccharids, a decrease of monosaccharids, and an increase of pentosans, while a high water content and lower temperatures are associated with a decrease of polysaccharids, an increase of monosaccharids, and a decrease of pentosans. Colin and Belval (2) could detect no carbohydrates in the leaves of the wheat plant except cane sugar and its hydrolytic products. Ver Hulst, Peterson and Fred (18) found that the pentosans of the leaves of corn approximated 19 per cent of the dry weight of the leaves from the time of tasseling to the dent stage, while the free pentoses amounted to only 0.52 per cent of the dry matter of the leaves during the same period.

## EXPERIMENTAL METHODS

### CULTURAL METHODS

The plants were grown under field conditions in rows 44 inches apart. The corn plants were thinned to a distance of 2 feet in the row and the sorghum plants to approximately 1 foot. The soil was kept free from weeds by hoeing, but no other cultivation was given. The moisture in the soil at the various periods of leaf sampling is given in Table I.

### COLLECTION OF MATERIAL

With but one exception, the experiments extended over a period of 24 hours, and the material for chemical analysis was collected at two-hour intervals during that time. The three uppermost fully unfolded leaves of the plants furnished the material in each case. After the leaves had been stripped from the plants, the distal third and the basal third of each leaf were discarded and the remaining portions, after the midribs had

been removed, were ground in a food chopper. The ground material was immediately placed in three times its volume of 95 per cent ethyl alcohol and sealed in glass jars. The time required to collect the material in this manner at each two-hour period approximated 15 to 20 minutes. At a convenient time the alcoholic material was placed in a hot-air oven and dried for 24 hours at a temperature of 100° to 105° C. The material was then placed in sealed glass jars until the chemical determinations could be made.

TABLE I.—Moisture content of the soil at the time of leaf sampling in 1916 and 1917 at Garden City, Kans., and in 1919 at Manhattan, Kans.

Date.	Percentage of moisture at a depth of—					
	1 foot.	2 feet.	3 feet.	4 feet.	5 feet.	6 feet.
Corn plot:						
July 20, 1916.....	10.0	14.7	19.0	21.9	23.6	24.2
August 1, 1916.....	7.3	11.8	14.0	18.1	21.4	22.0
Wilting coefficient.....	12.3	15.9	12.4	15.0	16.3	16.4
Milo plot:						
July 20, 1916.....	11.1	15.4	19.6	22.2	24.1	24.8
August 1, 1916.....	8.3	9.9	14.6	14.9	20.8	24.0
Wilting coefficient.....	12.2	13.4	15.1	14.3	15.4	15.6
Corn plot:						
July 25, 1917.....	11.2	11.6	18.4	19.3	23.1	22.3
Wilting coefficient.....	12.6	12.2	15.3	13.9	16.9	17.8
Milo plot:						
July 25, 1917.....	8.8	18.0	19.2	19.7	21.6	21.6
Wilting coefficient.....	12.3	15.9	12.4	15.0	16.3	16.4
Corn and sorghum plots:						
July 17, 1919.....	16.5	21.6	22.1	21.4	.....	.....
Wilting coefficient.....	12.2	12.5	12.1	12.7	.....	.....

#### ACTUAL AMOUNT OF CONSTITUENTS

Since the dry weight of a green leaf fluctuates through a considerable range during a 24-hour period, the analyses of leaf material expressed in percentage of dry weight only approximates the changes that occur in the leaf during a given period. The changes in the various constituents of the leaf may be followed more accurately by the determination of the dry weight of a unit of leaf area for each period that analyses are made, and from the percentage data for that period determine the actual amount of the various constituents in any unit of leaf area. The dry weight of a unit of leaf area at the close of each of the two-hour periods during the experiments was determined by the method previously described by the writer (10), and the amount of each of the constituents was expressed in grams per square meter of leaf.

#### CHEMICAL METHODS

The carbohydrates were estimated according to the methods of the Association of Official Agricultural Chemists.<sup>4</sup> In 1916 and 1917 the nonreducing sugars were determined by the difference between the total

<sup>4</sup> ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

sugars and the reducing sugars, but in 1919 the nonreducing sugars were estimated as sucrose. In the experiments of 1917 and in the case of corn in 1919, determinations were made of starch according to the official method of direct acid hydrolysis. In this method, however, the material estimated as starch includes the pentosans and other insoluble carbohydrate bodies which undergo hydrolysis and conversion into reducing sugars on boiling with hydrochloric acid. The results, while not indicating the actual amount of starch, do show the increase or decrease of the complex carbohydrates in the leaves during a 24-hour period.

#### DISCUSSION OF EXPERIMENTAL DATA

Three comparative experiments were conducted with Dwarf Yellow milo and Pride of Saline corn and one with Dwarf Yellow milo and Red Amber sorgo. In one experiment in 1917 Dwarf Yellow milo alone was under observation, while in 1919 in one experiment Pride of Saline corn was the only plant studied. During the three years, observations were thus made upon 10 different sets of plants. The two kinds of plants used in each of the comparative experiments were of the same age and were grown in alternate rows under the same cultural conditions, so that any differences that are observed in the changes of carbohydrates can be attributed to the specific differences of the plants under consideration. A general description of the plants at the time the material was collected is given in Table III. The evaporating power of the air during the time the plants were under observation was measured by Livingston porous cup atmometers, and the evaporation for each two-hour period during the time of each experiment is given in Table II.

TABLE II.—*Evaporation (in cubic centimeters) for the different periods of leaf sampling in 1916 and 1917, at Garden City, Kans., and in 1919, at Manhattan, Kans.*

Date.	Evaporation for period ending—											
	A. M.			P. M.						A. M.		
	8	10	12	2	4	6	8	10	12	2	4	6
1916.												
July 20.....	4.9	7.5	11.8	14.3	14.6	13.5	8.1	7.1	2.5	1.5	1.0	3.6
August 1.....	8.5	14.5	17.0	18.1	15.5	12.8	8.7	7.4	5.5	4.5	3.6	5.2
1917.												
July 25.....	2.9	6.3	8.0	9.3	9.7	8.6	8.5	4.1	2.7	1.3	1.6	0.6
August 3.....	2.6	7.1	9.3	10.5	11.5	11.6	9.9	.....	.....	.....	.....	.....
1919.												
July 3.....	6.1	9.8	12.6	15.0	15.5	14.5	8.3	4.0	3.8	2.9	1.8	.....
July 17.....	1.9	7.6	9.5	9.7	7.8	7.1	4.6	3.1	2.9	1.0	0.6	.....



TABLE III.—General description of plants used in leaf sampling in 1916 and 1917 at Garden City, Kans., and in 1919 at Manhattan, Kans.

Date of sampling.	Crop.	Height of plant.	General remarks.
1916.			
July 20-21.....	Corn, Pride of Saline.	3	8 fully unfolded leaves. Visible wilting 11 a. m. to 5 p. m.; guttation showing at 3 a. m.
	Sorghum, Dwarf Yellow milo.	2	Plants booting; no visible wilting; guttation showing first at 1 a. m.
Aug. 1-2.....	Corn, Pride of Saline.	4-5	12-14 leaves. Tassels just showing; leaves wilted from 11 a. m. to 4 p. m.; no guttation during night.
	Sorghum, Dwarf Yellow milo.	4	10-12 leaves. Plants in bloom; leaves slightly wilted from 1-3 p. m.; no guttation during night.
1917.			
July 25-26.....	Corn, Pride of Saline.	4	10 fully unfolded leaves; leaves not wilted during day; guttation showing at 3 a. m.
	Sorghum, Dwarf Yellow milo.	2	10 fully unfolded leaves; leaf of boot showing. No wilting during the day; guttation heavy from midnight until morning.
Aug. 3.....	Sorghum, Dwarf Yellow milo.	2-3	"Booting." No wilting of leaves during the day.
July 3-4.....	Corn, Pride of Saline.	5	9 fully unfolded leaves. Leaves not visibly wilted during the day; guttation heavy after 2 a. m.
July 17-18....	Sorghum, Dwarf Yellow milo.	3	8 fully unfolded leaves. No signs of wilting during day; heavy guttation after 1 a. m.
	Sorghum, Red Amber.	5	9 fully unfolded leaves. No wilting; heavy guttation after 1 a. m.

TABLE IV.—Daily variation of water, dry matter and carbohydrates in the leaves of *Pride of Saline* corn and *Dwarf milo* at Garden City, Kans., July 20 and 21, 1916

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
July 20.										
6 a. m. ....	26.3	29.6	73.7	70.4	2.75	3.68	1.87	2.44	0.88	1.24
8 a. m. ....	27.3	30.6	72.7	69.4	4.68	4.64	3.44	3.19	1.24	1.45
10 a. m. ....	28.1	31.3	71.9	68.7	5.13	5.24	4.01	3.94	1.13	1.30
12 m. ....	28.7	32.4	71.3	67.6	4.89	5.50	4.25	4.80	.64	.70
2 p. m. ....	29.4	33.0	70.6	67.0	6.03	8.27	4.83	6.98	1.20	1.29
4 p. m. ....	28.9	33.6	71.1	66.4	5.13	.....	4.44	.....	.69	.....
6 p. m. ....	28.1	32.8	71.9	67.2	4.58	6.55	3.65	5.25	.93	1.30
8 p. m. ....	26.8	31.5	73.2	68.5	3.31	4.22	2.54	3.28	.77	.94
10 p. m. ....	26.3	30.3	73.7	69.7	2.33	2.89	1.95	2.35	.38	.54
12 midn. ....	25.4	30.0	74.6	70.0	1.86	2.82	1.51	2.22	.35	.60
July 21.										
2 a. m. ....	24.3	28.4	75.7	71.6	2.19	3.16	1.60	2.20	.59	.96
4 a. m. ....	24.5	29.1	75.5	71.9	1.73	3.44	1.11	2.09	.62	1.35
6 a. m. ....	26.4	29.3	73.6	70.7	2.61	3.75	2.02	2.52	.59	1.23

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 20.										
6 a. m. ....	44.0	46.6	123.7	111.3	1.21	1.71	0.82	1.14	0.39	0.57
8 a. m. ....	45.3	47.2	120.7	107.0	2.12	2.18	1.56	1.51	.56	.67
10 a. m. ....	46.7	48.5	119.5	106.5	2.39	2.54	1.87	1.91	.52	.63
12 m. ....	47.3	49.9	117.7	104.5	2.31	2.74	2.01	2.39	.30	.35
2 p. m. ....	48.8	52.5	117.3	106.7	2.94	4.34	2.36	3.66	.59	.68
4 p. m. ....	48.5	54.2	119.5	107.2	2.49	.....	2.15	.....	.34	.....
6 p. m. ....	47.5	52.8	121.8	108.7	2.17	3.46	1.73	2.77	.44	.69
8 p. m. ....	46.4	52.0	127.3	113.4	1.34	2.19	1.03	1.71	.31	.48
10 p. m. ....	45.0	50.3	126.7	116.2	1.05	1.45	.88	1.18	.17	.27
12 midn. ....	43.0	49.7	126.5	116.1	.80	1.40	.65	1.10	.15	.30
July 21.										
2 a. m. ....	41.2	47.9	128.9	120.9	.90	1.51	.66	1.05	.24	.46
4 a. m. ....	41.7	46.7	128.7	119.5	.72	1.61	.46	.98	.26	.63
6 a. m. ....	44.2	48.1	123.5	116.5	1.15	1.80	.89	1.21	.26	.59

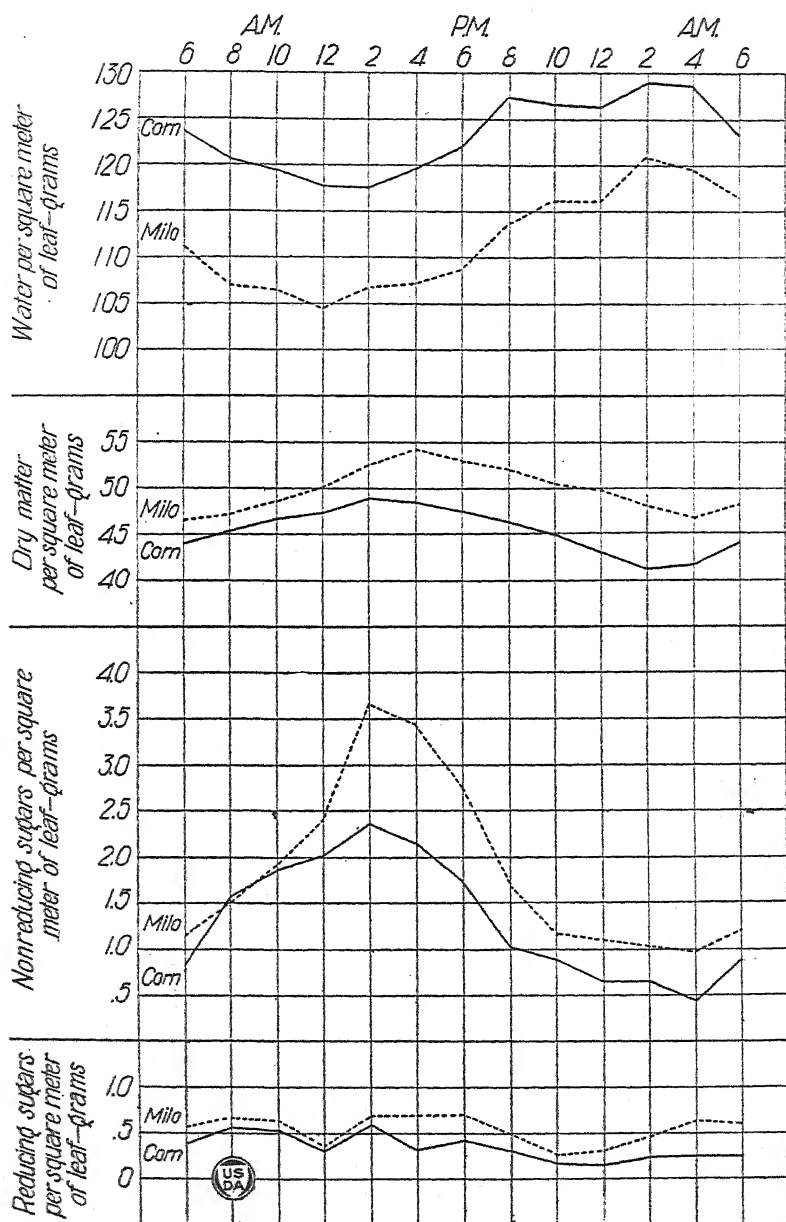


FIG. 1.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during July 20 and 21, 1916.

TABLE V.—Daily variation of water, dry matter, and carbohydrates in the leaves of *Pride* of *Saline* corn and *Dwarf* milo at Garden City, Kans., August 1 and 2, 1916

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
Aug. 1.										
6 a. m. ....	28.5	32.7	71.5	67.3	1.52	2.38	1.12	1.89	0.40	0.49
8 a. m. ....	29.7	34.4	70.3	65.6	3.49	3.33	3.23	2.45	.26	.88
10 a. m. ....	31.4	35.8	68.6	64.2	5.17	5.20	4.58	3.93	.59	1.27
12 m. ....	32.5	37.4	67.5	62.6	6.33	6.52	5.21	5.09	1.12	1.43
2 p. m. ....	33.1	38.2	66.9	61.8	5.68	5.62	4.71	4.65	.97	.97
4 p. m. ....	31.1	37.7	68.9	62.3	5.38	6.68	4.74	5.14	.64	1.54
6 p. m. ....	29.4	36.1	70.6	63.9	5.49	5.45	4.87	4.63	.62	.82
8 p. m. ....	28.9	34.8	71.1	65.2	1.80	3.71	1.22	2.70	.58	1.01
10 p. m. ....	27.7	33.9	72.3	66.1	3.39	3.39	2.77	2.58	.62	.81
12 midn. ....	26.7	32.3	73.3	67.7	2.88	3.24	2.03	2.02	.85	1.22
Aug. 2.										
2 a. m. ....	26.8	32.2	73.2	67.8	2.64	3.35	1.95	2.20	.69	1.15
4 a. m. ....	27.2	31.2	72.8	68.8	2.30	.....	1.72	.....	.58	.....
6 a. m. ....	27.1	32.6	72.9	68.4	3.01	3.47	2.33	2.38	.68	1.09

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

Aug. 1.										
6 a. m. ....	49.1	50.8	123.3	104.7	0.75	1.21	0.55	0.96	0.20	0.25
8 a. m. ....	50.0	53.4	118.6	102.0	1.74	1.78	1.61	1.31	.13	.47
10 a. m. ....	51.4	55.7	112.4	99.9	2.66	2.90	2.36	2.19	.30	.71
12 m. ....	51.3	58.3	106.9	97.9	3.25	3.80	2.68	2.97	.57	.83
2 p. m. ....	51.4	61.0	104.2	99.0	2.92	3.43	2.42	2.84	.50	.59
4 p. m. ....	51.7	61.5	115.0	102.0	2.78	4.11	2.45	3.16	.33	.95
6 p. m. ....	51.0	60.2	122.7	106.6	2.80	3.28	2.48	2.79	.32	.49
8 p. m. ....	50.2	58.0	123.7	108.7	.90	2.15	.61	1.57	.29	.58
10 p. m. ....	49.1	57.8	128.6	112.7	1.66	1.66	1.36	1.49	.30	.47
12 midn. ....	47.7	55.6	131.1	116.6	1.37	1.80	.97	1.12	.40	.68
Aug. 2.										
2 a. m. ....	47.3	54.9	120.2	115.6	1.25	1.84	.92	1.21	.33	.63
4 a. m. ....	46.6	52.1	124.9	115.0	1.07	.....	.80	.....	.27	.....
6 a. m. ....	47.1	54.2	127.2	117.4	1.42	1.88	1.10	1.29	.32	.59

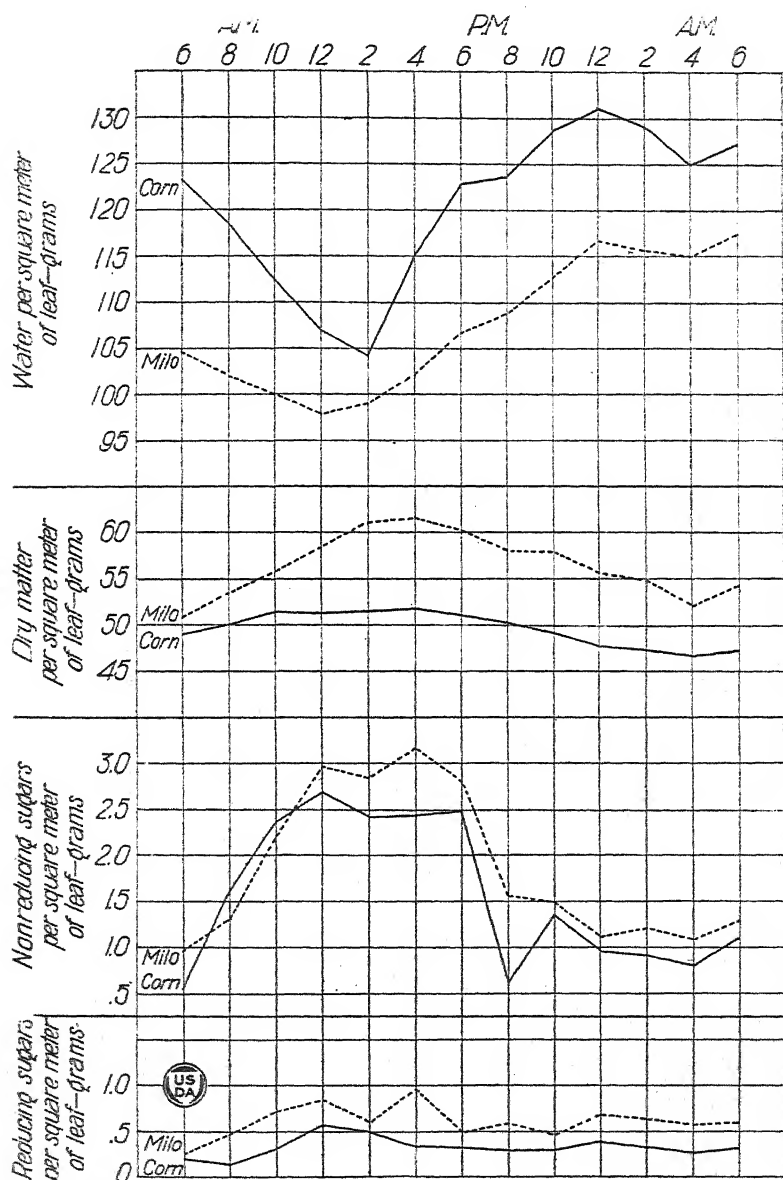


FIG. 2.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during August 1 and 2, 1916.

TABLE VI.—Daily variation of water, dry matter, and carbohydrates in the leaves of *Pride of Saline* corn and *Dwarf milo* at Garden City, Kans., July 25 and 26, 1917

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.		Starch. <sup>a</sup>	
	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.	Corn.	Milo.
July 25.												
6 a. m. ....	25.3	28.6	74.7	71.4	3.18	4.21	1.33	0.86	1.85	3.35	21.95	20.67
8 a. m. ....	26.1	30.1	73.9	69.9	3.80	5.10	1.91	2.34	1.89	2.76	20.41	19.54
10 a. m. ....	27.4	31.2	72.6	68.8	5.65	5.74	3.58	3.08	2.07	2.66	21.71	19.98
12 m. ....	28.7	32.2	71.3	67.8	6.46	6.64	4.75	4.80	1.71	1.82	20.89	21.07
2 p. m. ....	29.4	32.6	70.6	67.4	6.37	7.49	4.89	6.14	1.48	1.35	22.17	22.98
4 p. m. ....	27.7	33.6	72.3	66.4	4.80	5.10	3.53	4.51	1.27	.59	23.51	23.29
6 p. m. ....	28.0	33.7	72.0	66.3	4.75	3.93	3.85	3.11	.90	.82	23.30	25.70
8 p. m. ....	25.8	31.7	74.2	68.3	1.56	3.35	1.21	2.73	.35	.62	23.42	26.82
10 p. m. ....	25.3	30.3	74.7	69.7	1.11	1.53	.81	1.19	.30	.34	23.29	23.63
12 midn. ....	24.7	29.0	75.3	71.0	1.87	4.40	.77	2.24	1.10	2.16	24.50	25.34
July 26.												
2 a. m. ....	23.3	28.1	76.7	71.9	1.94	4.18	.61	2.05	1.33	2.13	23.66	23.51
4 a. m. ....	23.6	26.2	76.4	73.8	2.05	2.71	1.30	1.57	.75	1.14	21.53	21.55
6 a. m. ....	24.0	26.9	76.0	73.1	2.07	2.42	1.59	1.63	.48	.79	22.32	21.16

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 25.												
6 a. m. ....	47.5	45.3	140.5	113.6	1.51	1.91	0.63	0.39	0.88	1.52	10.43	9.36
8 a. m. ....	47.6	46.6	135.4	108.7	1.81	2.38	.91	1.09	.90	1.29	9.71	9.10
10 a. m. ....	49.9	49.1	132.3	108.3	2.82	2.82	1.79	1.51	1.03	1.31	10.83	9.81
12 m. ....	50.8	49.8	126.6	105.2	3.28	3.31	2.41	2.39	.87	.91	10.61	10.49
2 p. m. ....	52.9	51.1	127.3	106.0	3.37	3.83	2.59	3.14	.78	.69	11.73	11.74
4 p. m. ....	49.3	53.6	129.0	106.0	2.37	2.73	1.74	2.42	.63	.32	11.59	12.48
6 p. m. ....	51.9	55.4	133.6	109.3	2.46	2.18	2.00	1.72	.47	.45	12.09	14.24
8 p. m. ....	48.9	52.6	140.8	113.5	.76	1.76	.59	1.43	.17	.33	11.45	14.11
10 p. m. ....	48.5	52.1	143.3	120.0	.54	.80	.39	.62	.14	.18	11.29	12.31
12 midn. ....	46.8	50.1	143.3	123.0	.87	2.20	.36	1.12	.51	1.08	11.47	12.69
July 26.												
2 a. m. ....	44.0	47.9	145.3	122.9	.85	2.00	.27	.98	.58	1.02	10.41	11.26
4 a. m. ....	44.8	44.5	145.6	125.8	.92	1.20	.58	.70	.34	.51	9.64	9.59
6 a. m. ....	45.1	45.5	143.5	123.9	.93	1.10	.72	.74	.22	.36	10.06	9.63

<sup>a</sup> The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

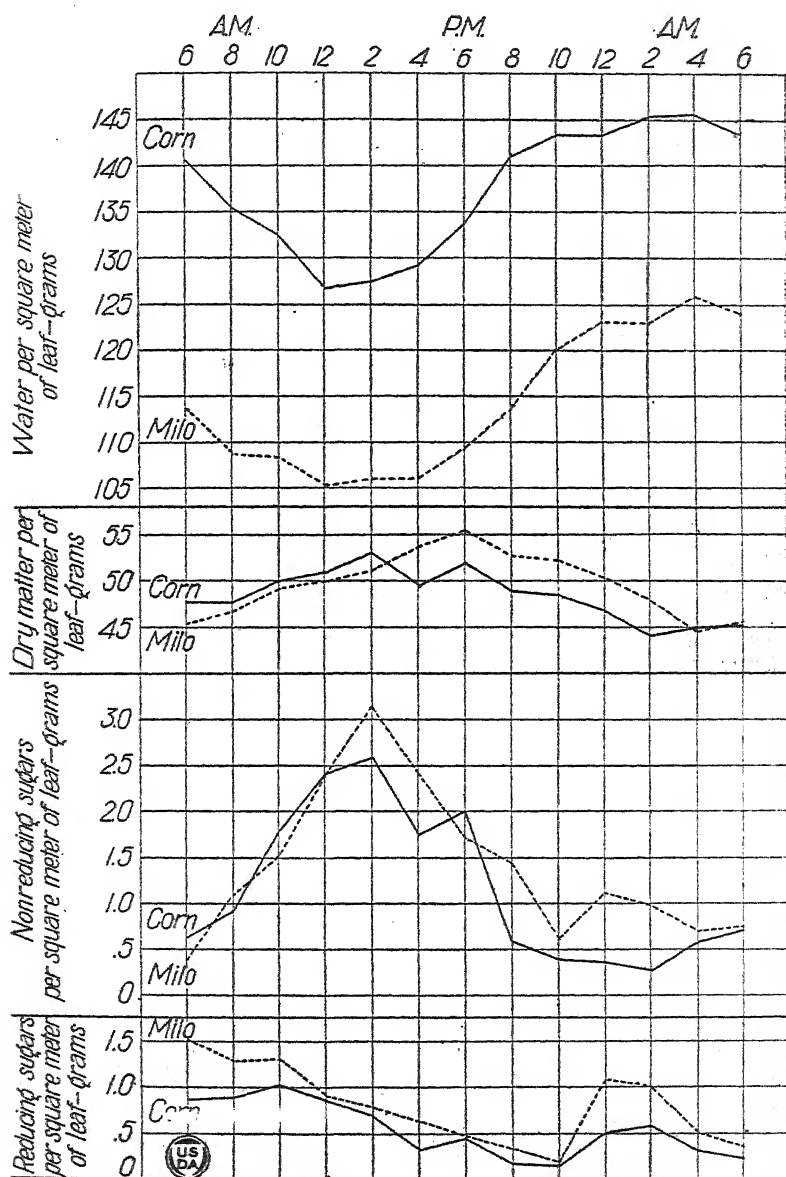


FIG. 3.—Graph showing the variation of the reducing and nonreducing sugars, dry matter and water in the leaves of Pride of Saline corn and Dwarf Yellow milo during July 25 and 26, 1917.

TABLE VII.—Daily variation of water, dry matter and carbohydrates in the leaves of Dwarf milo at Garden City, Kans., August 3, 1917

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.	Water.	Total sugars.	Nonreducing sugars.	Reducing sugars.	Starch. <sup>a</sup>
Aug. 3.						
6 a. m.....	31.3	68.7	2.25	1.37	0.88	20.86
8 a. m.....	32.9	67.1	4.62	2.17	2.45	19.51
10 a. m.....	34.0	66.0	5.92	3.91	2.01	21.91
12 m.....	35.8	64.2	6.77	4.87	1.90	21.44
2 p. m.....	36.2	63.8	5.59	4.33	1.26	22.23
4 p. m.....	36.4	63.6	4.25	3.25	1.00	23.29
6 p. m.....	36.2	63.8	2.82	2.25	.57	23.84
8 p. m.....	34.8	65.2	1.92	1.58	.34	24.23

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

Aug. 3.						
6 a. m.....	52.9	116.3	1.19	0.72	0.46	11.03
8 a. m.....	54.4	111.0	2.51	1.18	1.33	10.61
10 a. m.....	56.1	109.2	3.32	2.19	1.13	12.29
12 m.....	59.3	106.4	4.01	2.89	1.13	12.79
2 p. m.....	60.9	107.5	3.40	2.64	.76	13.54
4 p. m.....	61.5	107.5	2.61	2.00	.61	14.32
6 p. m.....	62.5	110.2	1.76	1.41	.36	14.90
8 p. m.....	61.9	116.3	1.19	.98	.21	15.00

<sup>a</sup> The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.



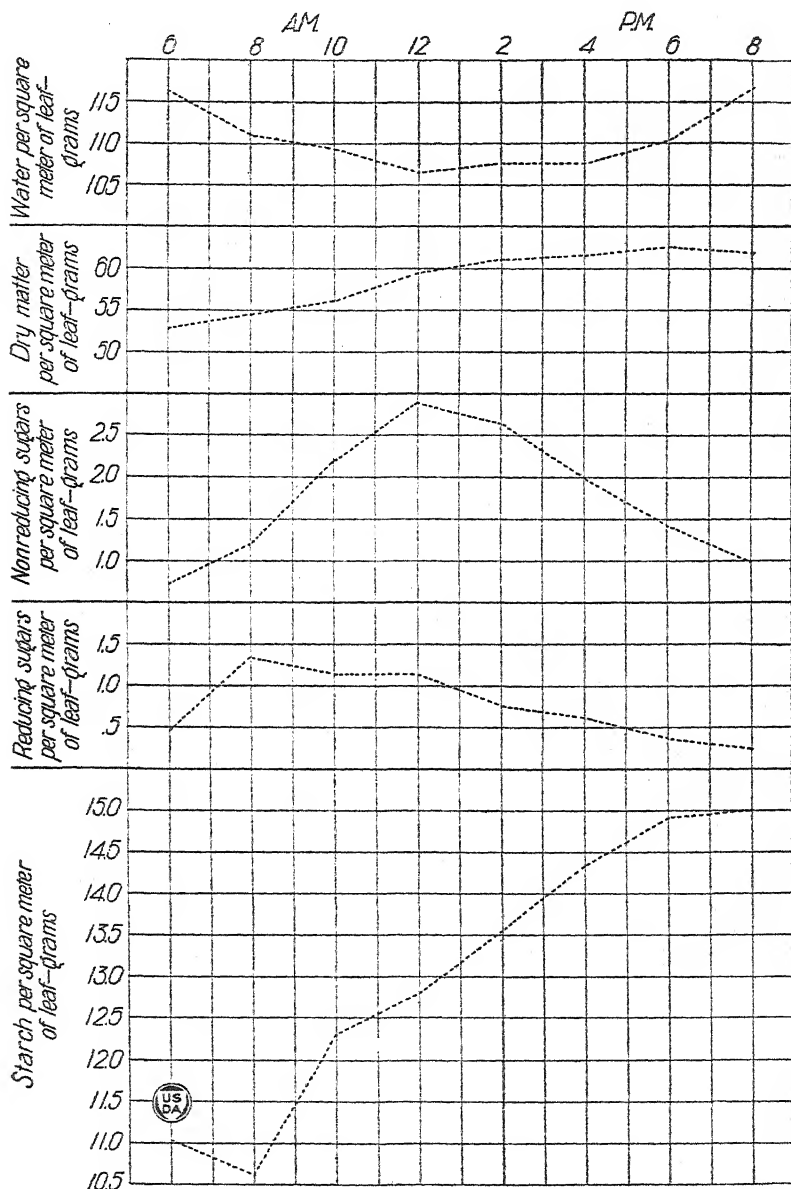


FIG. 4.—Graph showing the variation of the starch,<sup>5</sup> reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo during August 3, 1917.

<sup>5</sup> The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

TABLE VIII.—Daily variation of water, dry matter, and carbohydrates in the leaves of *Pride of Saline* corn, at Manhattan, Kans., July 3 and 4, 1919

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.	Water.	Total sugars.	Nonreducing sugars.	Reducing sugars.	Starch. <sup>a</sup>
July 3.						
7 a. m. ....	20.6	79.4	0.71	0.25	0.42	17.84
9 a. m. ....	21.2	78.8	1.01	.31	.65	18.72
11 a. m. ....	22.5	77.5	2.00	1.11	.79	19.75
1 p. m. ....	23.4	76.6	3.44	2.34	.90	21.12
3 p. m. ....	24.5	75.5	4.59	3.54	.81	22.34
5 p. m. ....	24.3	75.7	5.92	4.23	1.41	22.85
7 p. m. ....	24.2	75.8	5.14	3.22	1.68	22.22
9 p. m. ....	23.6	76.4	3.47	1.77	1.05	23.13
11 p. m. ....	21.9	78.1	1.99	1.19	.70	23.29
July 4.						
1 a. m. ....	20.7	79.3	1.60	1.04	.48	21.61
3 a. m. ....	19.8	80.2	.60	.28	.29	20.18
5 a. m. ....	19.0	81.0	1.51	1.02	.40	19.88

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 3.						
7 a. m. ....	39.3	152.2	0.28	0.09	0.16	7.01
9 a. m. ....	40.3	149.9	.40	.12	.26	7.54
11 a. m. ....	42.3	146.1	.85	.47	.33	8.35
1 p. m. ....	43.9	143.9	1.51	1.03	.39	9.27
3 p. m. ....	45.3	140.3	2.08	1.60	.37	10.12
5 p. m. ....	45.5	142.4	2.69	1.92	.64	10.34
7 p. m. ....	45.3	142.6	2.33	1.46	.76	10.07
9 p. m. ....	44.7	145.2	1.55	.79	.46	10.33
11 p. m. ....	43.0	153.8	.85	.51	.30	10.01
July 4.						
1 a. m. ....	42.2	162.5	.67	.44	.20	9.12
3 a. m. ....	40.0	166.0	.24	.11	.12	8.07
5 a. m. ....	40.6	173.3	.61	.41	.16	8.07

<sup>a</sup> The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis for the estimation of starch.

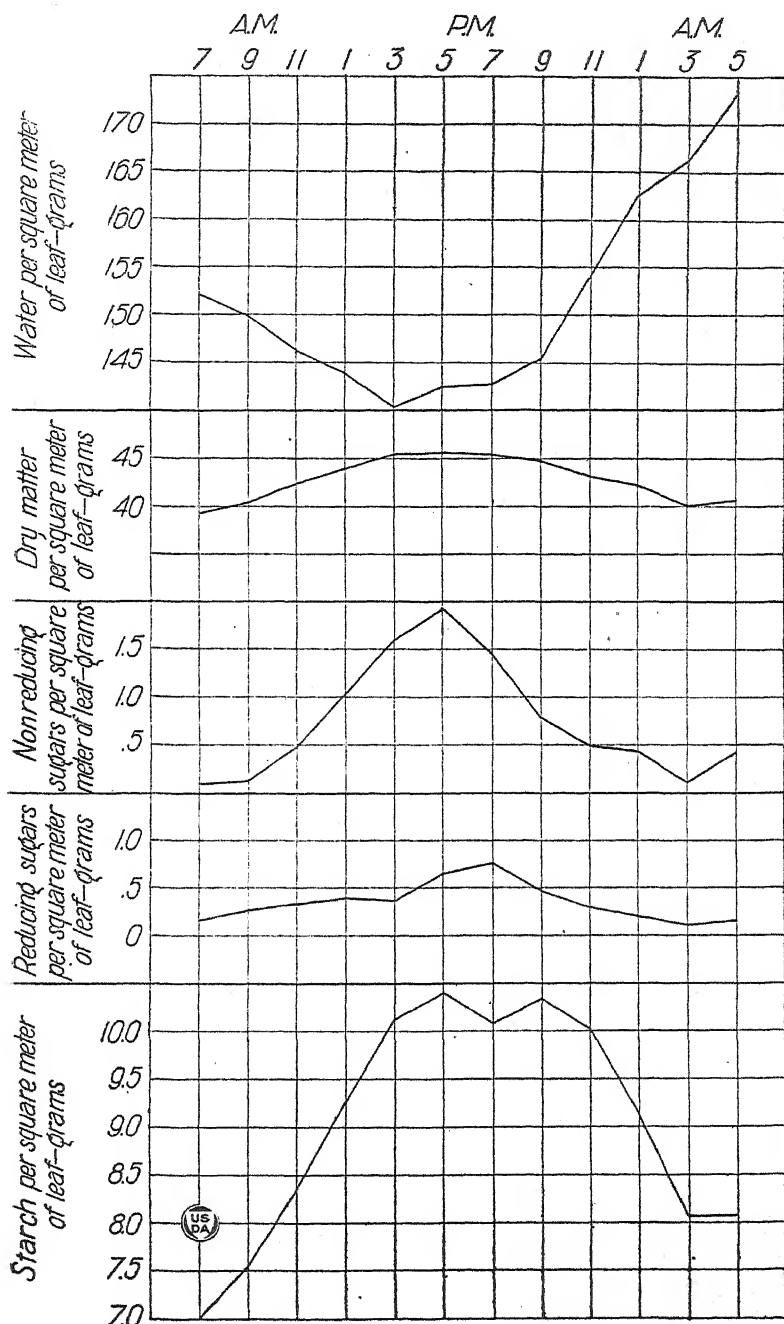


FIG. 5.—Graph showing the variation of the starch,<sup>6</sup> reducing and nonreducing sugars, dry matter, and water in the leaves of Pride of Saline corn during July 3 and 4, 1919.

<sup>6</sup> The term "starch" as here used includes, besides the starch, the pentosans and other insoluble carbohydrates that undergo conversion into reducing sugars on boiling with hydrochloric acid according to the official method of acid hydrolysis.

TABLE IX.—Daily variation of water, dry matter, and carbohydrates in the leaves of Dwarf milo and Red Amber sorgo at Manhattan, Kans., July 17 and 18, 1919

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.
July 17.										
7 a. m. ....	22.4	21.4	77.6	78.6	1.74	2.28	0.83	0.76	0.82	1.41
9 a. m. ....	24.2	23.3	75.8	76.7	2.53	3.16	.88	1.41	1.53	1.61
11 a. m. ....	24.6	25.0	75.4	75.0	3.34	4.20	1.08	2.18	1.18	1.84
1 p. m. ....	26.0	26.0	74.0	74.0	4.06	5.17	1.85	2.82	2.02	2.01
3 p. m. ....	26.4	26.7	73.6	73.3	4.11	5.25	2.43	2.50	1.48	2.53
5 p. m. ....	25.6	25.9	74.4	74.1	2.17	4.88	1.38	2.36	.68	2.30
7 p. m. ....	25.3	25.5	74.7	74.5	2.81	5.49	1.97	3.30	.70	1.94
9 p. m. ....	24.9	24.0	75.1	76.0	2.33	3.57	1.25	2.66	.97	1.34
11 p. m. ....	25.0	24.5	75.0	75.5	2.13	2.13	.82	.78	1.21	1.25
July 18.										
1 a. m. ....	24.4	23.5	75.6	76.5	1.77	2.05	.28	.72	1.41	1.22
3 a. m. ....	23.4	22.9	76.6	77.1	2.13	1.86	.79	.48	1.24	1.29
5 a. m. ....	22.2	21.6	77.8	78.4	2.14	1.38	.86	.16	1.16	1.15
7 a. m. ....	21.0	20.5	79.0	79.5	2.07	1.32	.60	.16	1.38	1.10

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 17.										
7 a. m. ....	41.1	40.6	143.0	150.0	0.71	0.93	0.34	0.40	0.34	0.57
9 a. m. ....	42.4	41.2	133.5	136.0	1.07	1.30	.37	.58	.65	.66
11 a. m. ....	43.0	43.4	132.0	130.6	1.44	1.82	.85	.95	.51	.80
1 p. m. ....	45.2	44.6	128.7	127.1	1.83	2.30	.84	1.25	.91	.90
3 p. m. ....	46.5	46.6	129.8	128.4	1.91	2.45	1.13	1.16	.69	1.19
5 p. m. ....	45.7	45.5	133.3	130.6	.99	2.22	.63	1.07	.31	1.05
7 p. m. ....	45.4	45.3	134.3	132.4	1.27	2.49	.80	1.49	.32	.88
9 p. m. ....	44.4	43.1	134.6	139.9	1.03	1.54	.55	.80	.43	.58
11 p. m. ....	45.3	43.8	136.3	135.5	.96	.93	.37	.34	.55	.55
July 18.										
1 a. m. ....	44.4	43.0	138.2	140.4	.78	.88	.12	.31	.63	.52
3 a. m. ....	43.3	42.6	142.1	144.2	.92	.79	.34	.20	.54	.55
5 a. m. ....	42.3	41.3	148.9	150.1	.91	.57	.36	.07	.49	.47
7 a. m. ....	40.0	39.0	150.9	151.3	.83	.51	.24	.06	.55	.43

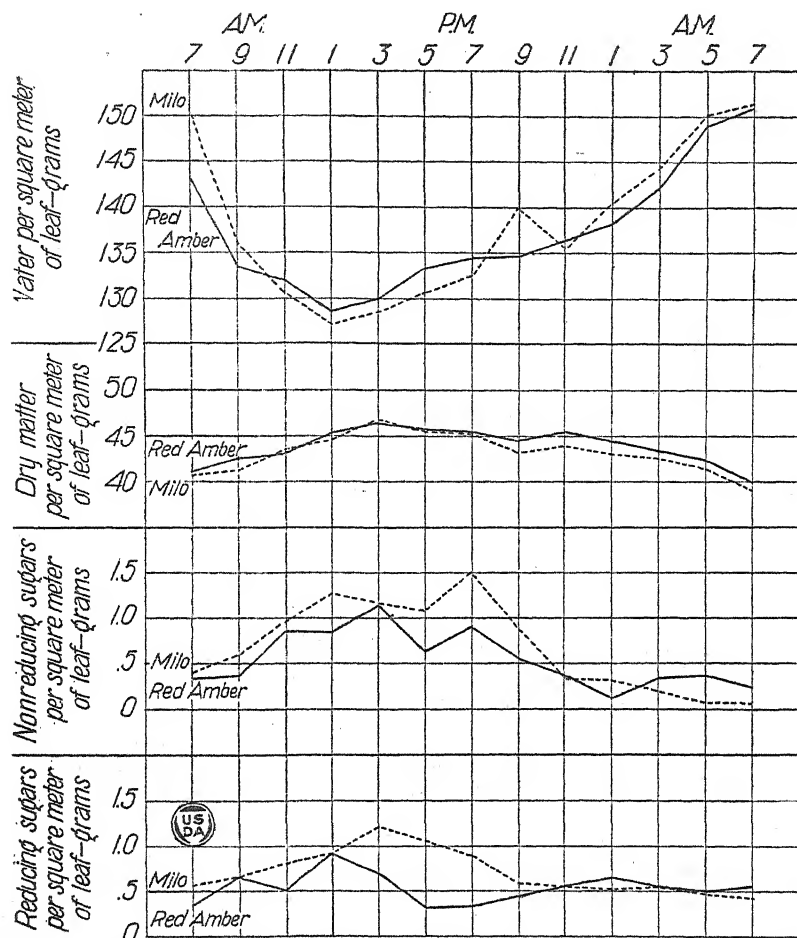


FIG. 6.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo and Red Amber sorgho during July 17 and 18, 1919.

The data in regard to the amount of water, dry matter, and carbohydrates obtained in each of the experiments are shown in Tables IV to IX. The results are expressed in percentages and in terms of the number of grams of constituents per square meter of leaf. The percentages of water and dry matter are calculated on a wet basis, while the percentages of carbohydrates are estimated on the amount of dry matter in the leaf. The changes in the amount of the constituents per square meter of leaf during each two-hour period of the experiments are shown by graphs in figures 1 to 6.

#### DRY MATTER AND WATER IN THE LEAVES

The daily variation of the water and dry matter in the leaves of corn and the sorghums has been reported in detail in a previous paper (10), so that only those facts will be mentioned that are essential to discussion of the variation of the carbohydrates of the leaves.

In the experiments the amount of dry matter in a given area of leaf was always greater for the sorghums than for the corn, and a microscopic

TABLE IX.—Daily variation of water, dry matter, and carbohydrates in the leaves of Dwarf milo and Red Amber sorgo at Manhattan, Kans., July 17 and 18, 1919

## PERCENTAGE OF THE CONSTITUENTS

Period ending—	Dry matter.		Water.		Total sugars.		Nonreducing sugars.		Reducing sugars.	
	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.	Red Amber sorgo.	Dwarf milo.
July 17.										
7 a. m. ....	22.4	21.4	77.6	78.6	1.74	2.28	0.83	0.76	0.82	1.41
9 a. m. ....	24.2	23.3	75.8	76.7	2.53	3.16	.88	1.41	1.53	1.61
11 a. m. ....	24.6	25.0	75.4	75.0	3.34	4.20	1.98	2.18	1.18	1.84
1 p. m. ....	26.0	26.0	74.0	74.0	4.06	5.17	1.85	2.82	2.02	2.01
3 p. m. ....	26.4	26.7	73.6	73.3	4.11	5.25	2.43	2.50	1.48	2.53
5 p. m. ....	25.6	25.9	74.4	74.1	2.17	4.88	1.38	2.36	.68	2.30
7 p. m. ....	25.3	25.5	74.7	74.5	2.81	5.49	1.97	3.30	.70	1.94
9 p. m. ....	24.9	24.0	75.1	76.0	2.33	3.57	1.25	2.06	.97	1.34
11 p. m. ....	25.0	24.5	75.0	75.5	2.13	2.13	.82	.78	1.21	1.25
July 18.										
1 a. m. ....	24.4	23.5	75.6	76.5	1.77	2.05	.28	.72	1.41	1.22
3 a. m. ....	23.4	22.9	76.6	77.1	2.13	1.86	.79	.48	1.24	1.29
5 a. m. ....	22.2	21.6	77.8	78.4	2.14	1.38	.86	.16	1.16	1.15
7 a. m. ....	21.0	20.5	79.0	79.5	2.07	1.32	.60	.16	1.38	1.10

## GRAMS OF CONSTITUENTS PER SQUARE METER OF LEAF

July 17.										
7 a. m. ....	41.1	40.6	143.0	150.0	0.71	0.93	0.34	0.40	0.34	0.57
9 a. m. ....	42.4	41.2	133.5	136.0	1.07	1.30	.37	.58	.65	.66
11 a. m. ....	43.0	43.4	132.0	130.6	1.44	1.82	.85	.95	.51	.80
1 p. m. ....	45.2	44.6	128.7	127.1	1.83	2.30	.84	1.25	.91	.90
3 p. m. ....	46.5	46.6	129.8	128.4	1.91	2.45	1.13	1.16	.69	1.19
5 p. m. ....	45.7	45.5	133.3	130.6	.99	2.22	.63	1.07	.31	1.05
7 p. m. ....	45.4	45.3	134.3	132.4	1.27	2.49	.89	1.49	.32	.88
9 p. m. ....	44.4	43.1	134.6	139.9	1.03	1.54	.55	.89	.43	.58
11 p. m. ....	45.3	43.8	136.3	135.5	.96	.93	.37	.34	.55	.55
July 18.										
1 a. m. ....	44.4	43.0	138.2	140.4	.78	.88	.12	.31	.63	.52
3 a. m. ....	43.3	42.6	142.1	144.2	.92	.79	.34	.20	.54	.55
5 a. m. ....	42.3	41.3	148.9	150.1	.91	.57	.30	.07	.49	.47
7 a. m. ....	40.0	39.0	150.9	151.3	.83	.51	.24	.06	.55	.43

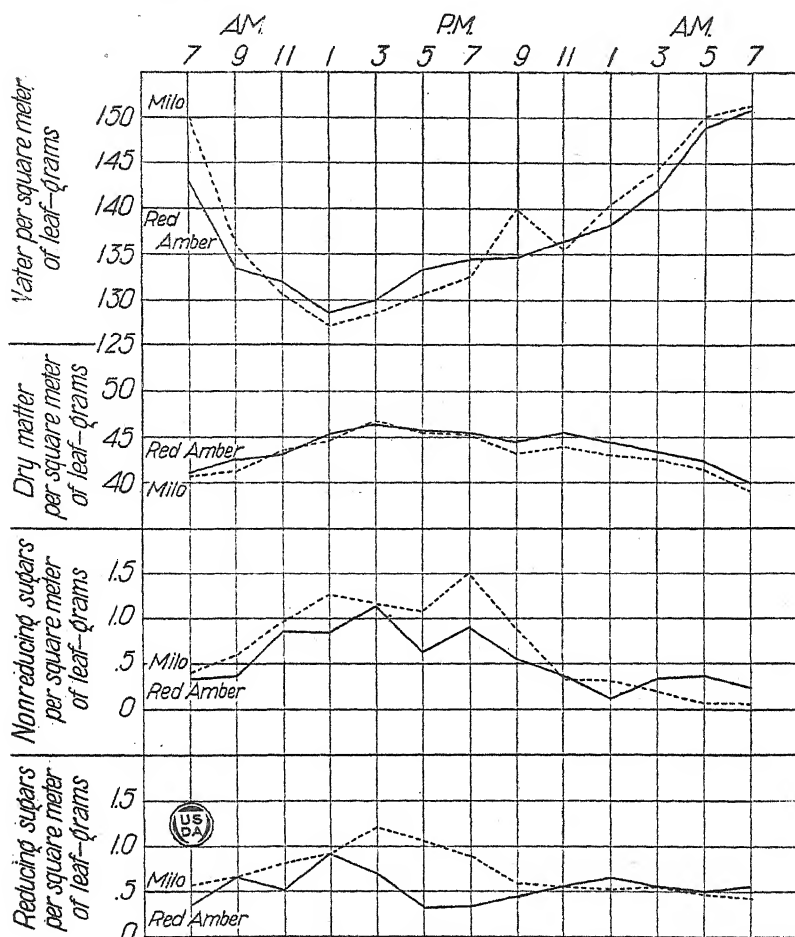


FIG. 6.—Graph showing the variation of the reducing and nonreducing sugars, dry matter, and water in the leaves of Dwarf Yellow milo and Red Amber sorgho during July 17 and 18, 1919.

The data in regard to the amount of water, dry matter, and carbohydrates obtained in each of the experiments are shown in Tables IV to IX. The results are expressed in percentages and in terms of the number of grams of constituents per square meter of leaf. The percentages of water and dry matter are calculated on a wet basis, while the percentages of carbohydrates are estimated on the amount of dry matter in the leaf. The changes in the amount of the constituents per square meter of leaf during each two-hour period of the experiments are shown by graphs in figures 1 to 6.

#### DRY MATTER AND WATER IN THE LEAVES

The daily variation of the water and dry matter in the leaves of corn and the sorghums has been reported in detail in a previous paper (10), so that only those facts will be mentioned that are essential to discussion of the variation of the carbohydrates of the leaves.

In the experiments the amount of dry matter in a given area of leaf was always greater for the sorghums than for the corn, and a microscopic

examination of sections of the leaves showed that the mesophyll cells of sorghum leaf are smaller, more numerous, and more compact than those of a leaf of corn. In the case of both corn and the sorghums, the amount of dry matter in the leaves begins to increase in the morning from 4 to 6 a. m., reaches a maximum at periods varying from 2 to 6 p. m., and then gradually diminishes until daylight the following morning, when the amount of dry matter again approximates that at the beginning of the previous day. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the increase in the amount of dry matter in the leaves during the day was always greater for the sorghum than for the corn. In each of these three experiments the maximum increase in dry matter per square meter of leaf was respectively 4.8, 2.6, and 5.4 gm. for the corn and 7.6, 10.7, and 10.1 gm. for the milo. If the materials used by the leaves in respiration are not considered, the increase in the weight of the dry matter of the leaves during any period of the day represents the difference between the amount of material manufactured by the leaves and the amount of material translocated from them during that time. It is not possible, therefore, to state definitely whether the greater increase in the dry weight of the leaves of milo as compared to the leaves of corn is due to a higher rate of production of materials by the leaves of sorghums or whether it is the result of a more rapid rate of translocation of the manufactured materials from the leaves of corn. The fact that the sorghums can increase the amount of dry matter in their leaves under climatic conditions that have prevented any increase in the amount of material in the leaves of corn would seem to indicate that the milo under the conditions of these experiments is able to manufacture materials in the leaves practically twice as rapidly as the corn.

Under the conditions of these experiments the amount of water in any given area of leaf was always lower in the sorghums than in the corn. The amount of water in the leaves of both corn and the sorghum begins to decrease anywhere from midnight to 5 a. m. and reaches a minimum from noon until 3 p. m., after which time the amount of water begins to increase until it again reaches a maximum the following morning. In the experiments with the sorghum the minimum amount of water in the leaves occurred at 12 noon in four cases and at 1 p. m. in two cases. In the four experiments with corn the minimum amount of water in the leaves occurred three times at 2 p. m. and once at 3 p. m. In only one case did the quantity of water in the leaves appear to be the limiting factor in the production of dry matter. In the experiment on August 1, 1916, the amount of dry matter in the leaves of corn reached a maximum at 10 a. m. and remained constant until after 4 p. m., when it began to decrease. The amount of water in the leaves at 10 a. m. was 112.4 gm. per square meter of leaf, and it continued to decrease until it amounted to only 104.2 gm. per square meter of leaf at 2 p. m. In none of the other experiments with corn did the amount of water in the leaves reach a minimum lower than 117 gm. per square meter of leaf. In all of the other experiments the amount of dry matter in the leaves of corn continued steadily to increase past the time of the minimum water content of the leaves, so that it would appear that the minimum amount of water that can be reached in the leaves of corn and not interfere with the process of photosynthesis is slightly above 112 gm. per square meter of leaf. The minimum amount of water observed in the leaves of Dwarf Yellow milo and Red Amber sorgo in these experiments was 98 gm. per square meter of leaf, but this minimum apparently in no way retarded the production of dry matter.



## THE CARBOHYDRATES OF THE LEAVES

## THE TOTAL SUGARS

The total sugars in the leaves of corn and the sorghums begin to increase between 4 and 6 a. m. and reach a maximum which varied in these experiments from 12 m. to 5 p. m. After the maximum was reached the sugar disappeared rapidly until 9 to 10 p. m., after which the decrease was very gradual until daylight again appeared. In five of the ten observations the maximum amount of sugar in the leaves occurred at the same time as the maximum amount of dry matter, while in the other five cases the maximum amount of sugar was reached at periods varying from one to six hours earlier than the maximum amount of dry matter in the leaves. There is no evidence from these experiments to show that there is any difference between the corn and sorghum in regard to the time of day at which the maximum amount of sugar and the maximum amount of dry matter may occur in the leaves. The fact that in some experiments the maximum amount of sugar in the leaves occurred earlier in the day than the maximum amount of dry matter shows that in these cases the increase in the dry weight of the leaves was due in part to the temporary storage of materials other than the sugars.

In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo, the maximum percentage of sugar in the leaves was respectively 6.03, 6.33, and 6.44 for the corn and 8.27, 6.68, and 7.49 for the milo. The average maximum amount of sugar in the leaves of the milo was thus about 1 per cent higher than in the leaves of corn. The maximum sugar content of the leaves during the day, expressed in grams per square meter of leaf, amounted to 2.94, 3.25, and 3.37 for corn and 4.34, 4.11, and 3.83 for milo, respectively, in each of the three successive experiments. The maximum increase in the amount of sugar in the leaves during the day was respectively 1.73, 2.50, and 1.86 gm. per square meter of leaf for the corn and 2.63, 2.90, and 1.92 gm. per square meter of leaf for the milo. A comparative experiment was conducted with Dwarf Yellow milo and Red Amber sorgo on July 17 and 18, 1919, but no significant differences were observed in regard to the behavior of the total sugars in these plants. Table X gives a summary of the more important facts concerning the changes in sugars of the leaves during the day.

## THE INSOLUBLE CARBOHYDRATES

In the experiment of July 25 and 26, 1917, the maximum amount of insoluble carbohydrates per square meter of leaf was reached at 6 p. m. in both the corn and milo, while the maximum sugar content occurred at 2 p. m. In the experiment with Dwarf milo on August 3, 1917, the maximum amount of sugar per square meter of leaf was reached at 12 m., but the maximum amount of insoluble carbohydrates did not occur until 8 p. m. In the observation on corn during the experiment of July 3 and 4, 1919, the maximum amount of sugar and insoluble carbohydrates per square meter of leaf was reached at 5 p. m. After the insoluble carbohydrates reached a maximum in the leaves, they showed little or no diminution in amount until about midnight, when they decreased rapidly until daylight.



The maximum increase in the dry matter of the leaves of corn and milo was always greater than the increase of the carbohydrates as determined by the methods used in these experiments. The case of Dwarf Yellow milo in the experiment of July 25, 1917, will serve as a concrete example of the above statement. On that date at 6 a. m. the dry matter in a square meter of leaf amounted to 45.3 gm. This increased gradually during the day until a maximum was reached at 6 p. m., when the dry matter amounted to 55.4 gm. per square meter of leaf. Thus each square meter of leaf increased in dry weight 10.1 gm. during the 12-hour period. The increase in sugars and insoluble carbohydrates from 6 a. m. to 6 p. m. amounted to 0.27 and 4.88 gm. per square meter of leaf, respectively, so that the total increase in dry weight in each square meter of leaf due to the carbohydrates amounted to 5.15 gm. There was thus an increase of 4.95 gm. of dry matter in each square meter of leaf that could not be accounted for by the increase of carbohydrates. In the several experiments the increase of dry matter during the day that could not be accounted for by the increase in sugar and insoluble carbohydrates during the same period varied from 7.4 to 53.7 per cent of the total increase of dry matter. Detailed information on this point can be obtained by consulting Tables VI, VII, and VIII. The undetermined material is evidently not protein, since unpublished data show that in the cases in question there is no increase in the absolute amount of nitrogen in the leaves during the day. The method used in obtaining the dry weight of a unit of area of leaf does not seem to be at fault, since the dry weight of a unit of area in all the experiments was approximately the same at the close of a 24-hour period as it was at the beginning. Since the samples used in determining the weight of a unit of leaf area at the beginning and at the close of an experiment were selected from rather widely separated portions of the leaf, it would seem that the structure of the leaf was uniform and that no appreciable error would be due to variations in the leaf samples that were used to determine the absolute amount of dry matter. The discrepancy observed between the total increase in dry matter in the leaves during the day and the increase in carbohydrates is due apparently to the fact that certain temporary storage products in the leaves are not detected by the methods used in determining the carbohydrates.

#### THE REDUCING AND NONREDUCING SUGARS

The nonreducing sugars in the leaves of the plants studied were, with the exception of the experiment with Dwarf milo and Red Amber sorgo, always in excess of the reducing sugars. The increase in the amount of the nonreducing sugars and the maximum point of increase of the total sugars is also the maximum point of increase of the nonreducing sugars. In the three comparative experiments with Pride of Saline corn and Dwarf Yellow milo the maximum increase in the nonreducing sugars in the leaves during the day amounted to 1.54, 2.13, and 1.96 gm. per square meter of leaf for the corn and 2.52, 2.01, and 2.75 gm. for the same leaf area in the case of the milo. In the same three experiments the maximum increase in the reducing sugars amounted to only 0.20, 0.37, and 0.18 gm. per square meter of leaf in the case of the corn and 0.12, 0.70, and 0.00 gm. for a like area of leaf in the case of the milo. In the experiment with milo on August 3, 1917, and with corn on July 3 and 4, 1919, the maximum increase in the reducing sugars in the leaves during the day was respec-

tively one-half and one-third of the increase of the nonreducing sugars. The highest ratio of the reducing sugars to nonreducing was that observed in the comparative experiment with Dwarf Yellow milo and Red Amber sorgo on July 17 and 18, 1919, when the maximum increase in the nonreducing sugars during the day was 1.09, and 0.79 gm. per square meter of leaf, respectively, for the milo and sorgo, while the maximum increase in the reducing sugars for an equal leaf area amounted to 0.62 gm. for the former and 0.57 gm. for the latter.

By consulting figures 1 to 6 it will be seen that the graphs representing the changes in the reducing sugars in the leaves of corn and the sorghums during the day are very irregular and that the time of the maximum increase in the amount of the reducing sugars very seldom if ever coincides with the time of the maximum amount of the nonreducing sugars. No significant differences were observed between corn and the sorghums in regard to the relationship between the reducing and the nonreducing sugars in their leaves. The observations made in these experiments in regard to the behavior of the nonreducing and the reducing sugars in the leaves of corn and the sorghums during the day and night are in accord with those of Brown and Morris (1), Parkin (11) and Davis (3, 4, 5) who worked, respectively, with the leaves of the nasturtium, snowdrop, and the mangold and potato. Since the nonreducing sugars increased so markedly in the leaves during the day and then decreased during the night, while the reducing sugars remained almost constant, these investigators concluded that the nonreducing sugars were the first sugars formed in the leaves in photosynthesis. It would seem, however, that it can not be determined definitely from chemical analyses whether the marked increase in the amount of the nonreducing sugars in the leaves during the day is due to the fact that they are the primary sugars of photosynthesis or to the fact that they are formed from the more simple sugars and accumulate in the leaves during the day as temporary storage products.

#### SUMMARY

In order to determine the changes of the carbohydrates in the leaves of corn and the sorghums during the day, analyses were made of material collected at two-hour intervals from the leaves of Pride of Saline corn, Dwarf Yellow milo, and Red Amber sorgo grown under identical or similar field conditions during the summers of 1916, 1917, and 1919. Ten sets of plants were under observation and the more important data obtained were as follows:

(1) The amount of dry matter in a given area of leaf of the sorghums studied was always greater than a like area of leaf of the corn. The dry matter in the leaves of both plants began to increase at daylight, reached a maximum at periods varying from 2 to 6 p. m., and then gradually diminished until daylight the following morning. In the comparative experiments with corn and milo the maximum increase in dry matter per square meter of leaf during the day was approximately twice as great in the leaves of milo as in the leaves of corn.

(2) The amount of water in a unit of leaf area was always greater in the leaves of corn than in the leaves of the sorghums. The water content of the leaves of both plants began to decrease anywhere from midnight to 5 a. m., reached a minimum from 12 m. until 3 p. m., and then began to increase until a maximum was reached the following morning.

In the case of corn the amount of water seemed to become a limiting factor in the production of dry matter when it reached a minimum of about 112 gm. per square meter of leaf. The water content of the sorghum leaves reached a minimum of 98 gm. per square meter of leaf, but this minimum apparently in no way retarded the production of dry matter.

(3) The total sugars in the leaves of the plants under observation began to increase between 4 and 6 a. m., reached a maximum which varied from 12 m. to 5 p. m., and decreased gradually from that time until daylight the following morning. In the 10 observations the maximum amount of sugar in the leaves occurred in five cases at the same time as the maximum amount of dry matter, while in the other five cases the maximum sugar content was reached at periods varying from one to six hours earlier than the maximum amount of dry matter. There is no evidence to show that there is any difference between corn and the sorghums in regard to the time of day at which the maximum amount of sugar and dry matter may occur in the leaves.

(4) The insoluble carbohydrates were estimated as starch according to the official method of acid hydrolysis and thus include besides starch the pentosans and other insoluble carbohydrates that are converted into reducing sugars by boiling with hydrochloric acid. The insoluble carbohydrates thus estimated generally reached a maximum later in the day than the sugars and after they had reached a maximum showed little decrease until about midnight, after which they decreased rapidly until daylight. The total increase in the dry matter of the leaves during the day could not be accounted for by the increase in the sugars and insoluble carbohydrates during the same period. The increase in the total sugars and insoluble carbohydrates in the leaves during the day only approximated from 46 to 92 per cent of the total increase in the dry matter of the leaves for the same period.

(5) The nonreducing sugars in the leaves of the plants studied were, with the exception of the experiment with Dwarf milo and Red Amber sorgo, always in excess of the reducing sugars. The nonreducing sugars increased markedly during the day and decreased during the night, while the reducing sugars, as a rule, showed very little increase, and the amount present at the different periods of the day was very irregular. The maximum increase in the reducing sugars in the leaves during the day in the case of corn and milo only amounted to from one-tenth to one-third the increase in the nonreducing sugars. No significant differences were observed between corn and the sorghums in regard to the relationship between the reducing and nonreducing sugars in their leaves.

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# JOURNAL OF AGRICULTURAL RESEARCH

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## STUDIES ON THE POTATO TUBER<sup>1</sup>

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### INTRODUCTION

A potato tuber is morphologically a modified stem with its axis greatly shortened and its lateral members only weakly developed, the latter forming what are known as the "potato eyes."

The tuber originates as a stolon from an axillary bud of the underground stem. At first clavate, the young organ soon assumes the shape characteristic of the variety. After a brief period of radial expansion, rapid apical growth becomes evident, new leaf scales are progressively differentiated from the vegetative cone, and in the axils of these scales new eyes and buds develop to maturity. Simultaneously with apical growth intercalary stretching occurs, whereby the spaces between the nodes widen and the spiral arrangement of the eyes becomes more distinct.

The close relationship between tuber and stem, already evident from the grosser morphological features, becomes incontestable when the anatomical structure and the ontogeny of the tissues are studied. In their early development both tuber and stem show practically the same organization; both have a bicollateral stele limited externally by a well developed cortex, internally by the pith. In their further development, however, each organ differentiates in accordance with the rôle which it is destined to play in the life of the plant. The stem, primarily an organ for support, develops a large amount of mechanical tissue and cells for water conduction—its vascular cylinder becomes hard and woody. The tuber, on the other hand, developing under the protective covering of the soil and destined to become an organ for storage, gives rise, as will be shown later, to a large amount of unspecialized parenchyma tissue. The different rôle of these two organs as indicated by their structural make-up is destined conversely to affect their outward appearance; that is, the stem will grow to a considerable length and remain slender, while the tuber will expand laterally as the flow of organic food material, constantly moving downward from the leaves, furnishes the Bausteine for new cells and tissues and for reserve starch which fills the cells as soon as they differentiate and mature.

### STRUCTURE OF THE STOLON PRIOR TO TUBER FORMATION

A median section through a stolon tip shows close behind the growing region three distinct tissue zones—the dermatogen, the procambium, and the fundamental meristem. These tissues become greatly extended and differentiated as the stolon tip develops into a tuber.

The dermatogen of the growing point becomes a single-layered epidermis. In surface view its young cells are square or hexagonal and more or less isodiametric. In maturing the cells become stretched in the

<sup>1</sup> Accepted for publication Nov. 24, 1923.

axial direction. Near the apex the cells are radially elongated; the tangential walls are short and equal in extent to the vertical walls. More distant from the growing point the tangential dimension of the cells increases, while the depth remains about the same. The tangential walls of the cells are slightly arched; the outer wall is always thicker than the inner, and is covered with a thin cuticle. Some of the epidermal cells are specialized to form guard cells of the stomates; others enlarge and divide to form cover hairs. Both stomates and hairs, however, are only sparingly found on the young stolon tip, and, because of subsequent development of the periderm, they have only a temporary existence. The young epidermal cells are devoid of chlorophyll and crystals, but rich in protein and solanin. The latter, a glucosidal alkaloid, is also found abundantly in the meristematic regions of the eyes at the time of renewal of growth in the spring.

The cells of the fundamental meristem enlarge without specialization, the region external to the procambium becomes the cortex, the zone inclosed by it becomes the pith.

The cortical parenchyma of the young stolon forms a comparatively broad band of tissue, about 9 cells wide and 345 microns in thickness. The typical cortical cell is rounded polygonal, slightly elongated or isodiametric; its two diameters are, on the average, 81 and 62 microns, respectively. Near the outer periphery the cells are sometimes collenchymatous and have a greater vertical extent. The cells at the inner margin are smaller and grade into the endodermis. Farther from the growing region the cells elongate vertically, but often remain isodiametric or become broader than deep. After the differentiation of the cortical cells from the meristem of the growing region, intercellular spaces form between them. These spaces are very narrow and appear in a cross section as small triangles.

Simultaneous with the formation of these intercellular spaces is the appearance of the first visible storage product—starch. The first starch, usually in the nature of fine round grains, is found in the cells near the procambium; later in larger deposits in other cortical cells. In the peripheral cells of the cortex, which are usually devoid of starch, protein crystals in the form of cubes of varying size are occasionally found; in the mature tuber they occur abundantly. Other cells of the cortex are conspicuous by a dark deposit which fills the entire lumen. This deposit is granular and consists of small crystals of calcium oxalate embedded in the nitrogenous remains of the protoplast of the cell.

The pith of the young stolon is small compared to the area of the cortex. The individual cells are vertically elongated and have a slightly smaller diameter than the cells of the cortex. Both pith and cortical cells are profusely pitted; the pits are minute and arranged in characteristic groups. Intercellular spaces of the nature found in the cortex appear close behind the growing region; starch and crystals, however, are deposited only at a later period.

The procambium, the progenitor of the vascular tissue, forms a hollow cylinder with small projections into the pith. It is composed of small, elongated, thin-walled cells with abundant protoplasm. In their further development these cells either merely enlarge or become highly specialized, forming the elements of the vascular system. The first specialization in the procambium becomes evident in the region of the procambium projections, for, if a cross section of the stolon be examined prior to tuber formation, several groups of vascular tissue with gaps separating



them are readily recognized. The groups of vascular tissue form bicollateral bundles. The xylem, occupying the middle of the bundle, stands out by the larger size of its elements and the secondary thickenings of the cell walls. About equidistant from the xylem internally and externally are small groups of thin-walled cells—the phloem. The outer phloem groups form a more or less continuous band, limited externally by the endodermis and separated from one another by parenchymatous cells of irregular size—the pericycle. The inner phloem groups are separated from the xylem by thin-walled cells which in their entirety make up the *Markkron*. Interjacent to the outer phloem and the xylem there is a single layer of procambium still in meristematic condition. It becomes the cambium of the bundles and later gives rise to secondary tissue.

The entire xylem of the bundles is limited to a few annular and spiral elements surrounded by unspecialized parenchyma. (Fig. 1.) The first formed protoxylem elements occur surprisingly close to the apex (Pl. 3) in a region in which there is much change and enlargement in the radial and tangential direction; these cells must therefore be able to accommodate themselves to increase in size and change in shape. This is made possible by their structure. The secondary wall is not laid down uniformly over the entire surface of the primary wall, but instead the secondary thickenings consist of rings or spirals. Elongation of the surrounding tissue causes the rings to be pulled farther apart, and as the stretching goes on the cells of the protoxylem may become completely flattened until the lumen is almost closed. The later formed protoxylem elements have thickenings in the form of close spirals and scalariform bands.

These elements are shorter than those formed earlier and are evidently developed from more mature initials which had completed their longitudinal growth. The end walls of the protoxylem elements are sloping, occasionally strictly transverse. The elements communicate with each other through a single large pore. (Pl. 2, E, D.)

The phloem groups consist of sieve tubes, their companion cells, and thin-walled parenchyma. The sieve tube is a long cylindrical cell with

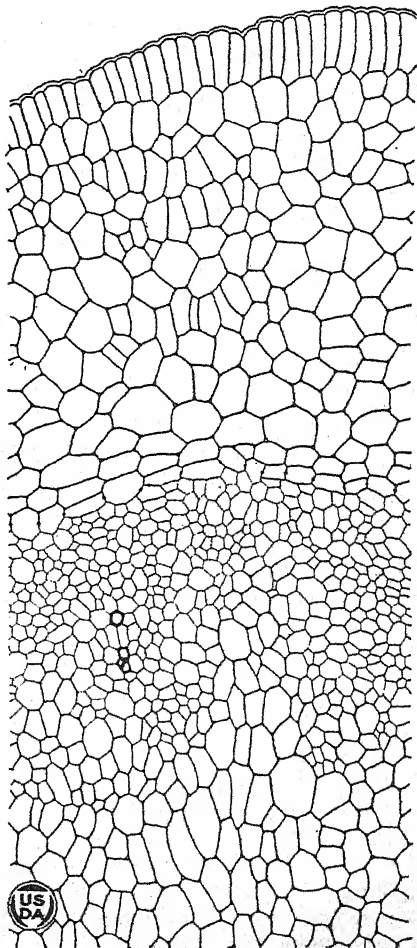


FIG. 1.—Cross section of stolon tip.  $\times 375$ . (Drawing is exact copy of photomicrograph.)

end walls strictly transverse. In diameter it varies between 2 and 9 microns, in length between 110 and 250 microns. Wherever branching of the bundles and anastomosis occur, the sieve-tube segments are short and somewhat broader. The sieve tube has a thin protoplasmic lining and albuminous content in which small starch grains are occasionally embedded. After fixation and staining, the content appears as a deep-staining shrunken mass—the slime plug. Its nature is not understood. The companion cell, a sister element of the sieve tube, has an even smaller diameter. It is formed from the sieve-tube mother cell by longitudinal division followed by transverse divisions. Only the companion cell retains its nucleus; that of the sieve tube disappears at a later stage. The companion cells are heavily pitted with the sieve tubes and with the phloem parenchyma. The cells of the latter differ but little from the larger sieve tubes in a cross-sectional cut. In longitudinal section the cells taper at both ends. They do not always remain undivided, but two to three cross walls are formed. The pits are very numerous and arranged in clusters, simulating sieve plates. But this type of pitting is very common in all parenchyma cells and by no means limited to the parenchyma of the phloem groups. In cross section the phloem parenchyma cells are polygonal with a diameter varying between 5 and 30 microns; in vertical direction they may attain a length up to 140 microns. The cells have a thin layer of protoplasm and a nucleus. They frequently contain a small quantity of starch and occasionally crystal sand. In primary phloem groups the proportion of sieve tubes and parenchyma cells shows much variation. Ordinarily the parenchyma cells are about twice as numerous as the sieve tubes. In the smaller groups, however, especially in the outer phloem, the representation of these two kinds of elements is often about equal. Compared with the stem the relative number of sieve tubes in a group is larger.

In close proximity to the primary phloem groups the phloem fibers differentiate. They are found either solitary, in the form of a tangential band, or in small clusters. The pits of these cells are much reduced, their slit-like openings either commonly parallel to the vertical axis or slightly inclined. They early develop a heavy secondary wall, whereupon their content disappears. The secondary wall, however, does not become lignified.

In the young stolon a cambium is noticeable only in the fascicular region. The cells are of the general shape and size of a tracheid. The end walls are pointed; the terminal walls follow an oblique tangential course. In radial section the sloping character of the wall is not evident.

Certain cells in the outer phloem region enlarge without specialization, making up in their entirety the pericycle of the stele. These cells are polyhedral, sometimes tangentially stretched. The young cells contain a large, spherical nucleus and parietal protoplasm. Before long, starch grains are deposited, and in the further growth of the tuber these cells enlarge and multiply rapidly, forcing the phloem groups farther and farther apart. In the mature tuber, as will be seen later, the tissue outside the vascular ring is mostly pericyclic tissue in which numerous phloem groups are scattered. The procambium cells between inner phloem and protoxylem develop in a similar manner. Their rapid multiplication causes a deflection of the inner phloem groups centripetally. As the tuber develops, the differences between cells of the *Markkron*

and of the pith gradually disappear until it becomes impossible to differentiate between these two tissues.

Between the vascular tissue and the cortex, there is differentiated in close proximity to the apex a special layer of parenchymatous cells—the endodermis. The cells of this layer are smaller than those of the cortex, are more regular, and lack intercellular spaces. On the radial walls there are hyaline swellings which stain red with phloroglucin and hydrochloric acid. These—the *Casparian strips*—though usually found as a narrow band along the radial walls often broaden out and cover a part of the tangential surface of the cell. At a later stage in the development of the endodermis a suberin lamella is deposited over the inner wall, which makes the cells more or less impermeable to dissolved substances and gases. As the young tuber enlarges, the endodermis is less readily detected. The cells with the radial dots become separated by cells resembling those of the cortex, and gradually this layer disappears altogether, and only vestiges of it are seen in the basal part of the tuber. By virtue of its structure the endodermis may be considered as a layer of cells which regulates diffusion between two regions of different pressure, and may be the cause of different pressure. Its primary function is probably to restrict passage of water and soluble substances to certain channels and to aid in the absorption of water (13).<sup>2</sup>

#### THE MATURE TUBER

A median longitudinal cut through a tuber shows two zones of tissue—a central pith with its lateral branches, and the vascular ring surrounded on either side by thick storage parenchyma, in which numerous small islets of phloem are embedded. The outer covering of the tuber is formed by the periderm, a new tissue which has replaced the epidermis of the tuber primordium.

The pith forms the central part of the tuber. It is broadest near the middle. In its course it gives off at intervals branches which in the form of laterally compressed hollow cylinders communicate with the lateral branches of the tuber—the eyes. The relative lengths of these branches and the angle which they form with the pith varies greatly, but is, nevertheless, closely related to the phyllotaxy and the steepness of the spiral formed by the eyes. The general appearance and the size of the pith and its branches also varies, but is more or less constant for a given variety. The pith either terminates abruptly in the region of the apical bud, or it branches out some distance below the apex, each branch communicating with one of the eyes of the apical eye cluster. In either case, however, the terminal bud forms a broad connection with the pith tissue—a fact which seems to be related to the time of renewal of growth.

The cortex forms a narrow band of tissue limited internally by an almost uninterrupted circle of phloem groups, externally by the periderm. In the young stolon, as has been previously noted, the cortex occupied a large area compared with the organ as a whole, but in the later development of the tuber the cortex adds but little new tissue, and hardly more than doubles the number of rows of cells in the radial extent.

The mature cells of the cortex, like those of the pith, are polyhedral, with a median diameter of about 180 microns. The periclinal walls are larger, because of the great peripheral growth of the organ. Compared

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 834-835.

to the cells of the pith, however, the cortex shows a greater degree of specialization. Some of the cells, especially in the region of the eyes, have greatly thickened walls, forming typical stone cells or sclereids. Not all varieties develop these stone cells. The peripheral cortical cells either abut abruptly on the periderm or form a transition zone three to eight cells wide. The cells in this transition region are smaller than the typical cortical cells, more elongated, and nearly rectangular. They are almost devoid of starch, but contain proteins, especially in the form of cubical crystals, and, in the case of colored varieties, also the pigment.

The epidermis of the tuber primordium has only a temporary existence. Anticlinal walls early appear in certain of the epidermal cells; later periclinal walls are also added. Simultaneously with the division of the cells of the epidermis, division walls also appear in the subepidermal layer. Soon a regular cork cambium becomes distinct, and cell division in this layer continues until a layer of tissue from 6 to 15 cells wide is produced, which assumes the protective function of the epidermis. The cork cambium, or phellogen, consists of a single layer of cells which divide tangentially and which constitute the inner row of daughter cells produced by the first division of the cells of the hypodermal layer. While most of the periderm arises from the phellogen derived from the hypodermis, the young tubers contain in addition a superficial periderm derived from the epidermis.

In the young tuber primordium the first periderm cells naturally appear in close proximity to the stem end, but soon the periderm extends over the entire tuber, and by the time the latter has reached only the size of a pea the characteristic number of rows of periderm cells has developed. The cork cambium remains active throughout the season, forming new cells to replace those which are sloughed off at the surface as the tuber expands. Loss and gain are thereby balanced, and the tuber shows through the various stages of its existence the normal thickness of skin, unless seasonal changes constitute a factor which changes the normal development.

The individual periderm cell is approximately brick shaped; its walls remain thin and later become suberized. The cells abut on each other, almost invariably without intercellular spaces. Small simple perforations are rarely observed (Pl. 10, K). The mature periderm cell is devoid of content. The younger cells contain small granules of tannin and the remains of the nucleus. Occasionally starch grains are found, but the latter are small, round, and do not show the striation characteristic of the large grains. In colored varieties the periderm cells contain the pigment, which, as has been previously noted, is also found in the peripheral cortical cells.

Simultaneously with the formation of the periderm the cells underneath the stomates, which are found widely scattered over the surface of the tuber primordium, begin to divide actively, producing a mass of loose tissue of roundish cells. Under favorable conditions these cells break through the epidermis and proliferate, becoming visible even to the naked eye as small white dots. Since the periderm is quite impervious to gases, this new structure—the lenticel (Pl. 8, E)—performs the function of aeration. This is facilitated by the shape of the cells and by the course of the intercellular spaces which run in radial direction.

Under certain abnormal environmental conditions it may happen that the periderm departs from the normal development. Such a condition was observed in Bliss Triumph potatoes grown in Ithaca, N. Y. The

tubers were, on the whole, normally developed; an examination of the periderm, however, showed that it differed from the normal in structure. The outer rows of cells were greatly hypertrophied (Pl. 8, E, D). The enlarged cells were found in all parts of the tuber, even covering the lenticels. Since other varieties grown under identical conditions showed a normal periderm, no satisfactory explanation was forthcoming for this abnormal formation.

If potatoes are cut and left in suitable surroundings, the surface of the cut becomes covered by a new "skin"—a wound periderm. The new cells appear in certain places and spread in all directions; the walls are laid down in a tangential plane. The cells resemble the normal periderm cells and, like the latter, perform the function of resisting evaporation and fungus attack. In this capacity the cells of the wound periderm appear to be even more effective than the normal periderm cells. Conditions which affect wound periderm formation have been the object of a great deal of investigation. The latest contribution, together with a review of the older literature, is by Edson and Shapovalov (4).

As the stolon enlarges to form the tuber, the ring of bundles begins to lose its definite arrangement in groups, and, since the development of parenchyma within the bundles tends to split up the separate groups still further, the entire vascular tissue becomes greatly spread out to such an extent that the vascular ring of the mature tuber can be compared only by analogy with the original one.

Cambium activity becomes evident even before the stolon tip enlarges to form the tuber, but the number of secondary elements thus added is conspicuously small, and only under favorable conditions does it attain the proportions shown in Figure 6. The secondary xylem cells are large, mostly porous, vessels with side walls heavily pitted (Pl. 2, E). The length of the elements varies greatly wherever the cells have been deflected from their normal course. Separating the radial rows of vessels are parenchyma cells homologous with the rays of the aerial stem. These cells rarely contain starch and their walls remain cellulose. The new phloem elements differ only in size from the cells of the primary tissue, and, like the former, they occur in small groups. The parenchyma cells surrounding these phloem groups are smaller than the normal storage cells, more elongated, and contain only fine-grained starch.

In their entirety the tissues so far discussed—namely, cortex, pith, and vascular ring—form only a small part of the tuber. By far the larger bulk of tuber tissue lying between cortex and pith and divided into two unequal parts by the narrow vascular ring consists of large polyhedral parenchyma cells in which small islets of phloem are embedded. What is this tissue morphologically? An inquiry into the ontogeny of the tuber should assist in arriving at a satisfactory solution (fig. 3).

The first change concomitant with tuber formation is an enlargement of the stolon in radial direction. This is brought about by successive cell division in the region of the pith. As a result of this localized growth, the vascular elements become deflected from their normally vertical course and are forced to continue in a more or less oblique direction. Simultaneous with division in the pith cells are changes in the peripheral cortex. To meet the dilation of the center of the stolon, the cells begin to divide radially whenever tangential stretching is unable to accommodate the constantly widening circumference of the organ. At the same time the cortical cells become filled with starch and occasionally dark cells of crystal oxalate appear among the white starch cells.

Soon after cell division in the pith is at its height and the above-described changes are going on in the cortex, the pericycle and the *Markkron* become the regions of greatest growth. The cells of the pericycle surrounding the primary groups of the outer phloem begin to divide and to enlarge rapidly, and as new cells are formed the older ones become filled with starch and soon become continuous with the cortex; in fact, they appear like wedges of the latter thrust into the zone of phloem tissue. However, their appearance, while the continuity of the endodermis still exists, permits of no other interpretation than that these cells are procambial in origin. Gradually, as the result of continuous cell increase in this tissue region, the cells of the endodermis fail to keep pace with the

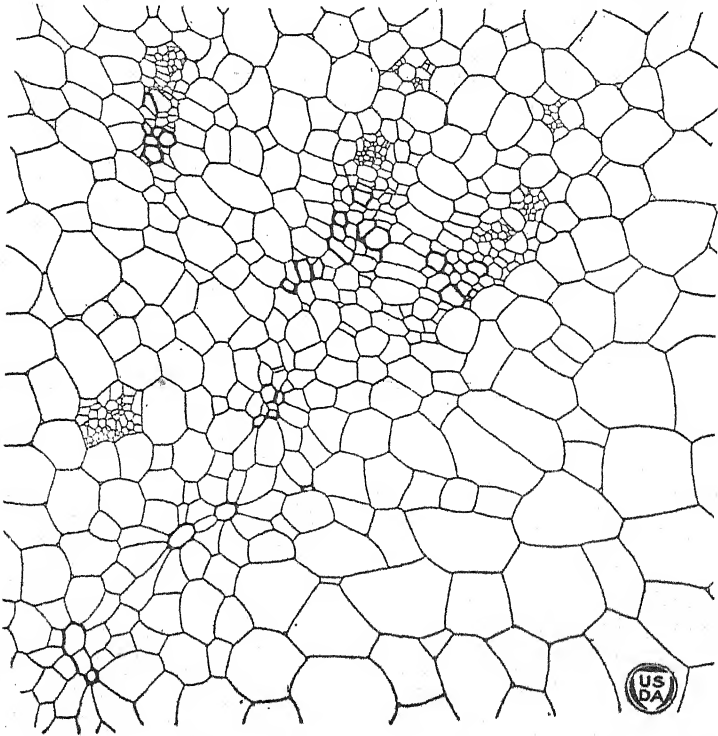


FIG. 2.—Cross section of vascular tissue of mature tuber.  $\times 75$ . (Drawing is a copy of photomicrograph.)

increasing circumference of the vascular tissue. Gaps appear here and there, gradually widening till finally all traces of this cell layer are lost in the starch-filled parenchyma, and only the outer circle of phloem groups indicates its former position. As a result of the activity of the pericyclic cells, the phloem groups become spread out; the individual strands become more widely separated from each other. New groups are formed as the tuber enlarges, and, grouped in larger or smaller constellations, they appear to the naked eye as dark dots surrounded by a halo of white starch cells. Detailed microscopic study shows the smaller groups to be made up of aggregations of the elements found in the ordinary primary phloem groups; i. e., a few sieve tubes with their companion cells and a number of conducting parenchyma elements. The cells surrounding

the phloem groups are smaller and more elongated than the storage cells and contain only small amounts of fine transitory starch.

To recapitulate: The tissue outside the vascular ring is composed of a narrow cortex analogous to the original cortex of the stolon and a much wider area of storage parenchyma containing numerous groups of phloem. This tissue is procambial in origin, although very much changed in its later development on account of the rôle which it is destined to play in the function of the tuber as an organ for storage.

Changes such as take place in the pericycle also proceed in the *Markkrona*. Rapid and continuous cell division causes here also a spreading

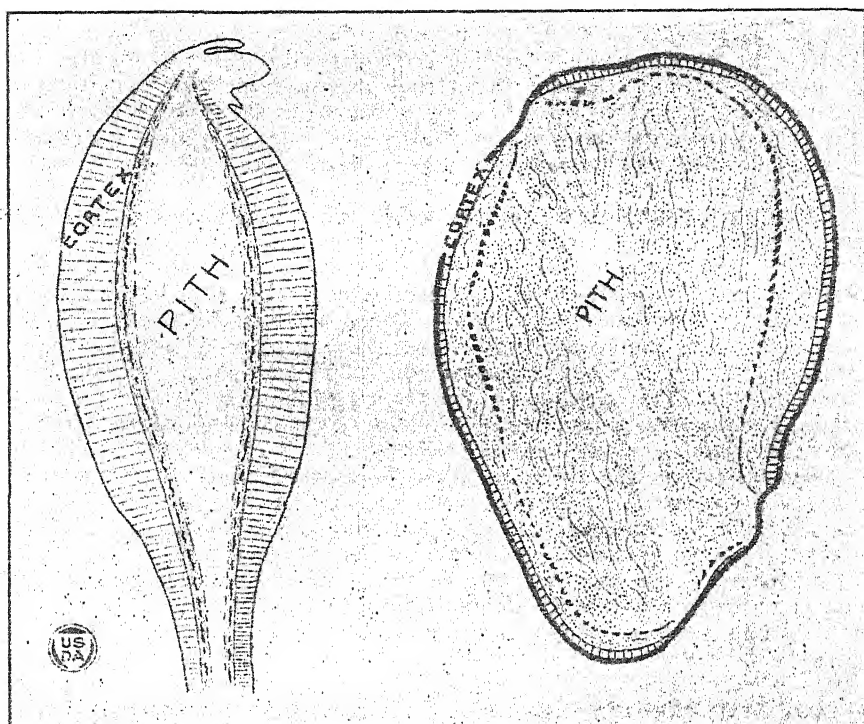


FIG. 3.—Diagrammatic drawings of radial section of stolon tip ( $\times 9$ ) and mature tuber (normal size) to show homology of tissues.

and deflection of the phloem groups centripetally. The activity continues till the tuber is mature. A certain amount of cell division also takes place in the region of the primary xylem whereby the protoxylem elements become more and more separated from the metaxylem, as seen in Figure 2. The band of tissue resulting from such growth, however, is inconspicuous.

In the mature tuber the vascular ring approaches the periphery in the region opposite the eyes, branching out in the buds and bud scales of the latter. The central pith is continuous with the eyes, as can easily be seen when a tuber only a few millimeters in diameter is examined. When the tuber enlarges and the distance between the peripheral eyes



and the center of the tuber naturally widens, the pith retains its connection with the eyes and its buds. Thus we see in the mature tuber branches radiating from the pith toward the periphery of the tuber (Pl. 1, A. B). In tangential section these branches appear as compressed hollow cylinders inclosing groups of phloem tissue. These pith branches, however, are not always symmetrical, since, on account of irregular growth activity, groups of phloem cells become more and more subdivided and spread out, causing a temporary or permanent breaking up and subdivision of these pith branches—a condition which becomes more conspicuous toward the periphery. In certain varieties the pith branches are large and uniform; in others, notably the Triumph, the branches are fine and almost indistinct; the cells are almost free from starch. The mass of tuber tissue inside the vascular ring is of two kinds—the storage parenchyma derived from cell division in the *Markkron*e and containing groups of internal phloem, and the pith with its lateral branches. The degree to which either of the tissues contributes to the formation of the tuber varies greatly with different varieties, but on the whole the pith occupies a smaller area than does the storage parenchyma.

The structure and growth of the potato tuber has been considered in earlier publications, the different writers giving their own interpretation.

In the growth of the tuber, as in any other organ, two factors are of importance—cell division and cell enlargement (Pl. 4, A, B, C). The latter factor is of special significance, since, as Esmarch (5) has shown, the cells of the young pith and cortex may increase to 4 times their original diameter, thereby increasing the volume to 64 times that of the young tissues. Cell division, however, is not equally rapid and extensive in the various tissues, and the investigators have been at variance as to the relative importance of each.

De Vries (20) considered most tuber tissue as potentially vascular and formed by a cambium, differing from the normal development in that almost all of the potential wood matured into thin-walled storage parenchyma. Conclusions from ontogenetic studies of the tuber, as developed in this paper, are in harmony with De Vries's view that the major part of the tuber is procambial in origin, but differ in attributing little activity to the cambium as such. The position of the protoxylem (fig. 2) in close proximity to the vascular ring shows how very little new tissue has actually been formed by the cambium.

Reed (15) showed that the tuber is built up by the activity of three tissues—pith, phloem parenchyma, and cortex. He clearly states that the cells between the internal phloem and the protoxylem begin to divide very early, causing a spreading out of the phloem groups. While this is in agreement with the writer's observations, it is difficult to assume that numerous cell divisions occur also in the parenchyma of the phloem groups and that through this activity a large amount of tissue is added to the growing tuber. It appears rather that the phloem parenchyma must be considered an integral part of the phloem groups and that its cells at maturity do not become storage parenchyma. The cell divisions which contribute to the increased size of the tuber must be sought in other tissues, and, as the writer has shown, most likely among the undifferentiated cells of the procambium.

Esmarch (5) regards the enlargement of the pith as the chief factor in tuber growth. He considers the internal phloem groups to be of



independent origin and in no way related to the vascular cylinder. However, the ontogeny of the vascular tissue, especially of the isolated groups of phloem and their subsequent development, excludes such a theory.

### THE POTATO EYE

The potato eye is a leaf scar with its subtended axil, which contains a suppressed lateral bud and undeveloped internodes. The eyes in their entirety show a definite arrangement in the form of a spiral (fig. 4), the direction of propagation of which is, according to the variety and the individual, either right or left. The arrangement of the eyes is thirteen ranked, since the fourteenth eye is over the first after five turns of the spiral. Each eye contains at least three buds arranged in the form of an obtuse triangle and protected by more or less conspicuous scales (fig. 5). Often, however, a larger number of buds are present which then form a secondary spiral to the left if the main spiral of the eyes is dextrorse, or conversely if the latter is sinistrorse.

The eyes of the tuber vary greatly in size and form, and the difference is further emphasized through the influence of environmental factors

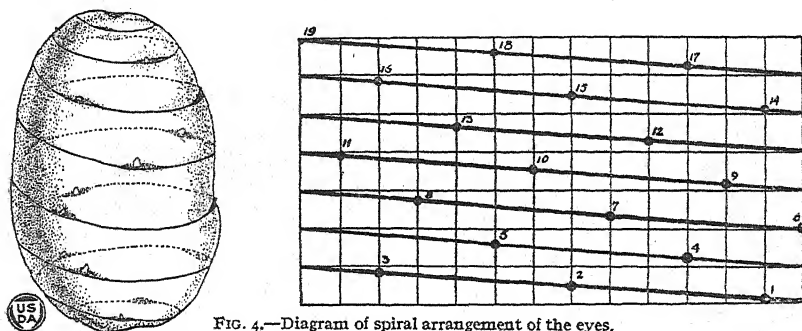


FIG. 4.—Diagram of spiral arrangement of the eyes.

which affect the development of the plant in general. There are shallow and deep-eyed varieties, and between the two extremes all intermediate types may be found. In a given tuber the lowest basal eye is small and inconspicuous; the eyes in immediate succession are larger and may protrude. After the first or second turn of the spiral the eyes become uniform and characteristic of the variety. The apical eye cluster is commonly not in direct line with the main axis, but excentric; the eyes composing it are small and contain fewer buds than those of the body of the tuber. There is, furthermore, a variation in the depth of these eyes. Some are very protuberant, but most are quite shallow.

A median longitudinal section of a stolon tip prior to tuberization shows the apical growing point with its protective scales and a number of buds developing in the axils of scaly leaves (Pl. 3). As the tuber enlarges, new buds are constantly differentiated from the growing apex, while the older ones gradually develop to maturity. In this growth and differentiation the scales, once very prominent, show a decided lag in development, and finally atrophy, leaving a mere scar, the "brow" of the mature eye. The size and form of the brow is correlated with the depth of the eyes. As a rule, the deeper the eye the more prominent the brow.

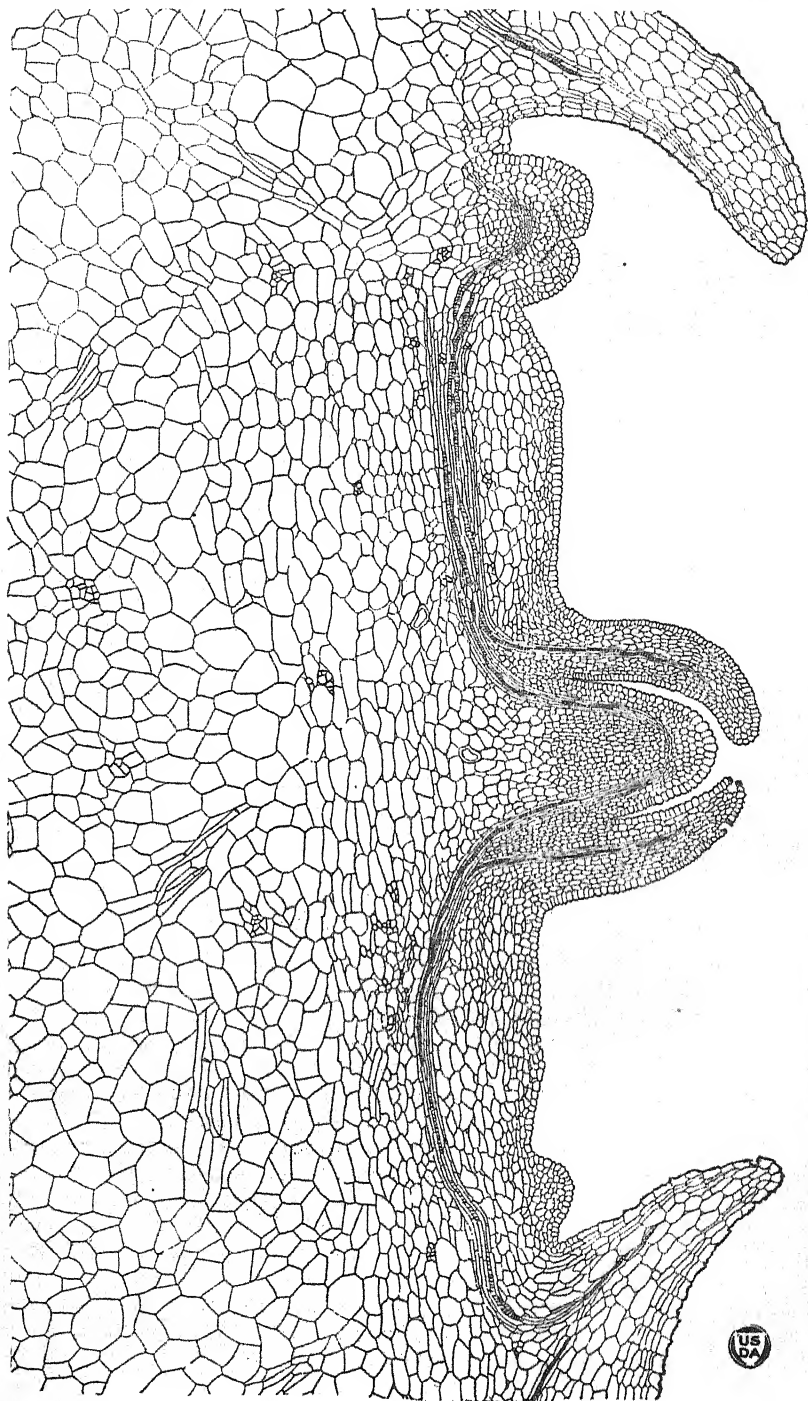


FIG. 5.—Drawing illustrating structure of potato buds and surrounding tissue.

In structure and tissue differentiation the resting bud resembles a stolon tip. The apical region, well protected by overlapping scales, is composed of a mass of cells rich in protoplasm. The walls are thin and of cellulose except for the outer wall of the epidermis, which is covered by a thin cuticle. Since the latter is devoid of a cellulose basis, as shown for other plants by Priestley, parasitic fungi which secrete cytolytic enzymes can penetrate the cuticle only by force of hydrostatic pressure. Where the bud merges into the tuber, differentiation into the characteristic tissue region takes place and the vascular tissue of the bud and bud scales joins the vascular ring of the tuber, which in the region of the eyes closely approaches the periphery. Like the vascular ring, the pith retains its connection with the lateral members of the tuber in that branches of the pith continue directly into the eyes. Since all the eyes thus maintain a connection with the central pith, sections through any part of the tuber show a system of such connecting strands of pith radiating from the center toward the periphery. The size and shape of these pith connections are similar in tubers of a given variety, and may, as will be shown later, serve as a group character.

With the termination of the growing season, the tuber has completed its development; the bulk of the tissue has matured and becomes filled with reserve materials, chiefly in the form of starch. Only small, restricted areas of the tuber, the buds, possess meristematic tissue, ready to resume life activity with the completion of the rest period and when external conditions permit of cell division and growth. Since the tuber is a shoot with indeterminate growth, the central bud is the youngest and should normally resume growth first. However, the prolonged state of dormancy which affects all eyes equally should have a leveling influence and remove whatever advantage the terminal eye may have possessed. Yet the earlier development of the apical buds is a fact, and to explain it various theories have been considered.

Franz (6), in his anatomical studies of the potato tuber, noticed that the terminal eye was shallow and not buried in cortical tissue; that the cells in the vicinity of the bud contained more water and were richer than elsewhere in protein; that the pith formed a direct and broad connection with the apical bud; and since the latter was also ontogenetically the youngest he concluded that the morphological and chemical differences sufficed to account for the earlier development of the terminal eye.

While the evidence brought forward by Franz may not be contested, it must be remembered, nevertheless, that the vascular tissue of the tuber, no matter whether it connects to lateral or terminal buds, is at best only weakly developed and that the repressive effect of a thick cortical tissue appears nowhere in evidence. The direct and more massive pith connection and the broader point of contact which the terminal eye forms with the vascular tissue are undoubtedly advantages which the lateral eyes lack. This fact is emphasized by the results of some experiments of Miss Fernald,<sup>3</sup> who found a rather definite correlation between the osmotic concentration of the tissues around the eyes and the tendency for these eyes to sprout. While Miss Fernald's results may explain why the basal eyes are inhibited, they in no way explain why the apical eyes get the earlier start. The morphological advantages which the apical eye possesses over the basal ones, as shown above, seem to offer the most reasonable explanation for the observed dominance.

<sup>3</sup>FERNALD, Evelyn I. THE INHIBITION OF BUD DEVELOPMENT AS CORRELATED WITH OSMOTIC CONCENTRATION. (Title.) *1st Amer. Assoc. Adv. Sci. Program 76th Meeting*, p. 93, 1922.

## CYTOLOGICAL STUDIES

NUCLEUS AND MITOTIC DIVISION.—Némec (11) studied the nucleus and division stages in the cells of the root, the stem apex, and wound periderm. He found noticeable differences in the formation of the achromatic figure in the cells of the wound periderm. In the normal division there accumulates around the nucleus at the beginning of prophase a fine granular substance, in which, at a later stage, two periplasts differentiate at the poles. As these periplasts enlarge, delicate fibers begin to develop from the apex and grow out toward the equator, where they unite to form the spindle. When the spindle is formed, the nuclear membrane disappears and the chromatin, which during prophase formed a peripherally disposed thread, differentiates into chromosomes which, after undergoing longitudinal cleavage, arrange themselves at the equator. The chromosomes form short thick rods; they number at least 36. The nucleolus contains a single central or several vacuoles in which small, cyanophyllous granules are precipitated in fixed material. The nucleolus disappears with the formation of the equatorial plate, although its residue sometimes persists during metakinesis. Often they appear again, in the vicinity of the poles, at the end of metakinesis. In the cells of the wound periderm the development of the achromatic figure differs primarily in that the spindle fibers are not developed from a hyaline periplast, but grow directly from the surface of the nucleus and parallel to the division axis. The nuclei of the cells of the wound periderm are at least 30 per cent larger than those of the normal cells, and the chromosomes may reach as high a number as 70.

Since we possess in the work of Némec an excellent account of the nuclear phenomena in the potato, it will suffice here to illustrate the principal division figures (Pl. 5) and proceed to a consideration of the types and structure of the nuclei in the various tissues, notably the phloem. In the apical meristem of the young stolon all resting nuclei are spherical (Pl. 5, A, B, and Pl. 7, C), with a mean diameter of about 12 microns. Each nucleus is composed of a large nucleolus surrounded by a hyaline sphere and a homogeneous nuclear substance in which chromatin granules of various sizes are embedded. The same type of nucleus, although often of larger dimensions, is found in all unspecialized parenchyma cells of the tuber. In the cells of the procambium and their derivatives the nuclei show a great variety of form and structure. They are often elongated with ends either blunt or pointed (Pl. 5, C-G and Pl. 7, A, B). Often they are rod-shaped, reaching a length of 30 microns or more. Normally there is one nucleolus to a nucleus; in the large and elongated forms, however, two or even three nucleoli may be present (Pl. 5, G).

The nucleus of the young sieve tube is large and fusiform. There is, as a rule, one nucleolus; occasionally one or two secondary nucleoli are found. As the sieve tube matures, the dense protoplasmic content, so conspicuous in the young element, becomes limited to a peripheral layer, while the nucleus itself gradually disintegrates. In the mature sieve tube, where the sieve plate and slime plug are easily recognized, a nucleus is no longer present. In a few instances the nucleus has been seen to persist in a more or less degenerate form even in the mature element.

The protoplasm of the sieve tube initial is granular and homogeneous, like that of other meristematic cells. At an early period, however,

certain structures differentiate out, which, because of their characteristic appearance, have recently been the cause of some misconception as to their nature and function. This peculiar type of sieve tube inclusion was first observed by Nelson (10) in the potato and other plants. The bodies were described as straight or twisted rods with pointed ends and occasionally bearing an attenuate process at one or both ends. The true value of the discovery was temporarily marred by an attempt to identify these bodies with certain flagellates and ascribing to them a probable rôle in the production of the mosaic disease. However, although these bodies were wrongly identified and their supposed connection with the mosaic disease was further invalidated by the fact of their occurrence in healthy as well as in diseased plants, their discovery is not without interest. An insight into their origin and nature was much to be desired, and some attention has been given to them in this morphological study of the potato tuber.

The bodies first become noticeable as light staining, looped or undulating bands along the inner margin of the cell or, more commonly, adhering to one side (Pl. 7, G). Often the bands are short, pointed and somewhat spirally twisted (Pl. 7, E, H; Pl. 6, C, B, E) abutting on the nucleus of the cell (Pl. 7, G) or lying next the end wall (Pl. 6, A, B, E). The bodies exhibit an extreme polymorphism, which is very pronounced within a single element (Pl. 7, F). Once formed they appear to retain their shape and change only in staining quality. They show at an early stage a strong affinity for acid fuchsin (Pl. 1, D) and safranin. When carefully stained with Haidenhains haematoxylin, they present distinctly a dark staining outer shell and a lighter interior (Pl. 6, D), in which are embedded dark granules. Old bodies may, however, stain uniformly black.

These structures usually originate in the cytoplasm of the sieve tube, yet in some cases their nuclear origin appears indisputable. They are abundant in the sieve tubes of the growing apex, but are also found in more mature tissue where new phloem groups are developing; they are typically a constituent of the young element, since they are not observed in cells with a well-developed sieve plate. Their elective power for acid fuchsin and their positive reaction in certain microchemical tests (Biuret, Millon) show them to be protein in nature. They are not typical protein crystals, but rather bodies homologous to those described by Strasburger (18, p. 196, Pl. 3) and Mrazek (9) for the Papilionaceae. They are undoubtedly concerned in the metabolism of the sieve tube, but their real significance leaves room for much speculation for which only comparative studies on an extensive scale can give a proper basis.

#### CHEMICAL CONSTITUTION OF THE TUBER

ORGANIC STORAGE PRODUCTS.—The most important reserve material of the tuber is starch, which is deposited soon after the stolon tip swells to form the tuber. The first starch is formed in the cells near the vascular tissue, commonly in the form of fine round grains. Somewhat later starch becomes noticeable in the cells of the cortex, and finally in the pith. The typical grains are ovate, about 40 to 100 microns long. In cross section the grains are spherical. While the small starch grains are more or less homogeneous, the large oval grains have an excentric hilum and show distinct striation. Compound starch grains are occasionally

observed in which each component has a hilum and striations of the nature found in the large individual grains.

Nitrogenous substances in various forms, as constituents of the protoplasm or dissolved in the cell sap, are found most abundantly in the cambium, the phellogen, and in the region of the buds; also in the form of crystals in the peripheral cells of the cortex. The protein crystals appear very early and can be found even in the tuber primordium. They increase rapidly in number and size as the tuber enlarges, so that in the mature tuber more than one may be observed in a cell. The crystals are always cubical in form, but their size varies greatly. The larger and more typical crystals have a diameter of 12 microns (Pl. I, C). Upon germination the cleavage products of the protein crystals form, according to Sorauer, the first food for the developing buds.

A small amount of fat is found in the periderm cells and in the peripheral cortex. Inulin is absent, but organic acids—citric, succinic—are present, lending the tuber an acid flavor which is apparent even without analysis. Sugar is not found in the mature tuber, but becomes abundant upon renewal of growth.

**CRYSTALS.**—Besides the typical protein crystals described above, two other types are commonly observed—calcium oxalate, in the form of crystal sand, found in special cells and observed first in the cortex of the young stolon. In the older tuber those cells often occur in vertical rows. The crystal sand consists chiefly of small crystals of calcium oxalate in the form of octahedrons and their derivatives. Together with the calcium oxalate there is always a protein matrix which remains after the crystals have been dissolved by acids. The crystal-bearing cells increase in number and appear not only in the cortex but also in the pith. In the mature tuber they disappear, but become evident again in large numbers in the region of the buds in the germinating tuber. In the peripheral cortical cells there are found occasionally crystals of basic calcium phosphate. These form four-sided prisms with pointed ends; however, they appear rarely in pure crystal form and often contain coloring matter. In time of their appearance the phosphate crystals are later than calcium oxalate. Sorauer considers calcium oxalate crystals a decomposition product of the carbohydrates, and the phosphate crystals a residue of protein metabolism.

**SOLANIN.**—When a section through an eye is treated with alcoholic phloroglucinol, it will be observed that the region of the buds stains a deep orange. The color is most intense in the epidermis and in the parenchymatous cells inside the vascular cylinder. Similarly, when a section is treated with concentrated sulphuric acid or ammonium vanadate with sulphuric acid (1 part of ammonium vanadate is dissolved in 1,000 parts of sulphuric acid, prepared by mixing 98 parts of sulphuric acid with 36 parts of water), the following color reaction is observed: Yellow, red-yellow, brown-red, dark red, violet, fading out. According to von Brehmer (2), who studied the distribution and migration of solanin in the potato, there is only one color change, from yellow-red to violet; all other colors merely indicate different concentrations of solanin. Von Brehmer found in his studies that solanin can not be extracted with water and is not held in combination by acids, and suggested that it is held in solution by colloidal proteins. Upon germination the solanin content diminishes in the peripheral cortex and becomes evident in the elongating shoot. Solanin is a glucoside which upon hydrolysis gives solanindin, galactose, rhamnose, and, as an intermediate product, a complex sugar.

It dissolves easily in hot alcohol, slightly in benzol, cold alcohol, and ether. Solanin is found in large quantities in the young tuber; in the mature tuber only in the region of the buds. Upon renewal of growth it begins to accumulate in larger quantities, and as it gradually disappears from the base of the tuber it becomes more evident in the shoot. The distribution and formation of solanin suggest that it is important in the metabolism of the growing plant. If germination is held back in the spring, solanin may accumulate in such quantities in the tuber, as von Brehmer has shown, as to be poisonous to man.

**TANNIN.**—One of the most conspicuous and interesting forms of cell inclusions is tannin. In the growing tip it occurs in two forms, as fine granules, notably in the older periderm cells, and in the form of spherical vesicles in the neighborhood of the buds (Pl. 8, A, C, and colored Pl. 1, C). The vesicles occur abundantly in the germinating tuber. They color blue with iron salts and black with osmic acid, and of themselves may be brown or blue. Their content is either a fine granular substance or a homogeneous brown fluid. The larger vesicles, which sometimes fill the entire cell, often contain a number of small globules within them. Sometimes the vesicles are of a lighter color, or even colorless; sometimes they form mere refractive drops. Sorauer (17), who made an extensive study of the nature and distribution of these bodies, observed that the white tubers contained mostly brown vesicles and that in colored varieties a mixture of brown and blue vesicles was found.

#### STRUCTURE OF THE STOLON WITH SPECIAL REFERENCE TO FOOD CONDUCTION

In the structure of the mature stolon the epidermis and the vascular tissue merit special consideration. In the tuber, as we have seen, the epidermis is replaced by a many-layered periderm, while in the stolon the epidermis is retained, but its outer walls and cover hairs become lignified. In the development of the vascular tissue strength has been sacrificed for efficiency in conduction. The xylem forms a continuous ring, composed mostly of large porous vessels. The area occupied by the outer and inner phloem is relatively greater than that in the stem. Sieve tubes are numerous and of a large diameter. The sieve tubes of the secondary phloem are especially large and measure as much as 20 microns in cross section.

An average-sized stolon bearing a medium-sized tuber had the following tissue areas (fig. 6 and Table I):

TABLE I.—*Relative areas occupied by the different tissues of the cross section of a mature stolon.*

Stolon tissues measured.	Area.	Per cent of total.
	<i>Sq. mm.</i>	
Cross sectional area of stolon.....	2. 130	100. 0
Cross sectional area of outer phloem.....	. 277	} 24. 2
Cross sectional area of inner phloem.....	. 239	
Cross sectional area of xylem.....	. 174	8. 2
Cross sectional area of pith.....	. 350	16. 3
Cross sectional area of cortex.....	1. 090	51. 2



Dixon (3) obtained similar figures for the phloem area of potato stolons. (A stolon 1.6 mm. in diameter had a cross sectional area of the phloem of 0.42 sq. mm.) From experimental evidence and theoretical considerations, Dixon concluded that organic substances could not pass through the phloem tissue in such amounts as must pass during the active growth of the tuber and that it is the xylem which conducts most of the food. However, in the stolon the xylem does not exceed the phloem area, or is, at best, only twice the area of the sieve tubes proper. In the tuber the xylem is greatly reduced, while the phloem is correspondingly increased. For the stolon it may be granted that xylem and phloem share equally in the transport of organic substances; in the tuber, however, the phloem and the extensively pitted storage parenchyma are probably the channels of translocation.

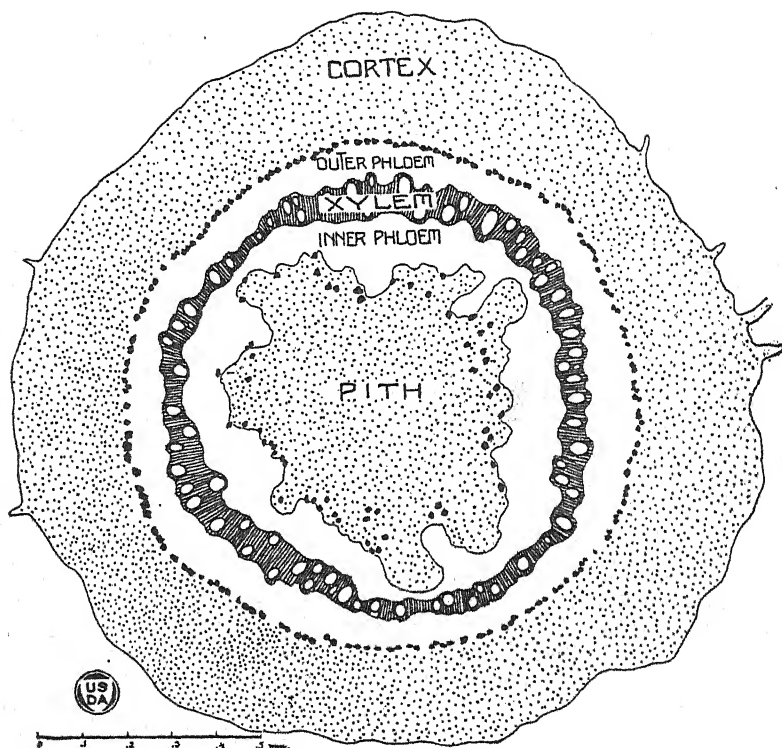


FIG. 6.—Diagrammatic drawing of cross section of mature stolon, giving the relative areas occupied by the different tissues.

#### COMPARATIVE STUDIES

Differences in the external morphology of the tuber, such as shape, color, type of eyes, have been used in the classification of potato varieties. It was found, however, that climatic and soil conditions greatly impaired the diagnostic value of these characters, and therefore it became necessary to consider the plant as a whole in the classification scheme. An anatomy of the potato tuber would be incomplete without at least an attempt at a study of such internal characteristics as might aid in the task of grouping potato varieties or establishing their relationship. Yet, if such a study is to be fruitful, the effect of environment, so potent in



shaping the external form of the tuber, must be taken into consideration, not for one, but for a number of generations.

In the present study only one generation of plants grown in different localities (the States of Maine, New York, Pennsylvania, and Colorado) was included. The suggestive results obtained from this preliminary study appear to warrant further investigations along this line.

In this comparative study the following terms, as defined, are used for convenience only, and are not considered as having strict morphological significance:

Pith: The central region of the tuber extending as an axial strand from stem end to apex.

Medulla: Lateral connections which the pith forms with the buds.

Cortex: Tissue between cambium and periderm.

Periderm: The potato skin.

Stone cells: Cortical cells which possess a thick, lignified wall.

PITH AND MEDULLA.—The pith forms the axial core of the tuber. It varies in thickness, attaining its greatest diameter a little below the center. At intervals branches in the form of compressed hollow cylinders leave the pith, take a more or less oblique vertical course, and connect each with one of the eyes. These medullary branches attain various proportions, and in cross section exhibit configurations which are more or less characteristic of the variety. The medullary branches vary in both form and distinctness. To be sure, young growing tubers have a less distinct pith and medulla than mature tubers, especially if the latter have been in storage for a certain time; yet when examined under identical conditions the tubers show differences which are distinct and form a constant feature of the different varieties. In tangential section the medulla branches appear in the form of open or closed horseshoe-shaped areas, and as the radial cut is approached the relation of these areas to the pith and eyes can clearly be seen.

#### CLASSIFICATION OF VARIETIES

Medulla distinct and large, antler-shaped; pith commonly large.—*Cobbler, Rural* (Pl. 9).

Medulla distinct, either large or small; pith large.—*Ohio, Up-to-date, McCormick* (Pl. 9).

Medulla branches distinct and fine; pith usually small.—*Triumph, Green Mountain, Rose* (Pl. 9).

Medulla branches fine; pith either large or small.—*Michigan, Hebron, Pearl* (Pl. 9).

Medulla branches indistinct; pith varying in size.—*Burbank*.

This classification, based on median cross-section view of tubers, is at best only a tentative one. While the medulla configurations are fairly constant, the pith is very variable, especially in tubers which are off type. Environmental factors appear to be of subordinate importance, since marked local variations are very pronounced; in other words, tubers grown in Maine and New York, for example, show no more variation than those from a given field in either State.

CORTEX.—The cortex forms a narrow band of tissue limited externally by the periderm, internally by the cambium of the vascular ring. In the region of the eyes the cortex becomes narrow and disappears completely as the vascular tissue branches out into the buds of the eye. Aside from the variations in the region of the buds, the width of the cortex throughout the tuber shows a more or less pronounced fluctuation.

These differences may be local or affect an entire side, so that one longitudinal half of the tuber has a broad cortex, the other half a narrow one. External irregularities also tend to affect the width of the cortex; but an external swelling may not always indicate a correspondingly wider cortex; on the contrary, this tissue in such regions is often narrower (Pl. 9).

## CLASSIFICATION OF VARIETIES

Wide cortex (8-11 mm.).	Medium cortex (6-7 mm.).	Narrow cortex (3-6 mm.).
Hebron. Michigan. Early Ohio. McCormick. Up-to-date. Burbank (seedling).	Cobbler. Rose. Burbank.	Green Mountain. Triumph. Rural. Spaulding Rose.

The varieties which are most likely to possess an irregular cortex are: McCormick, Hebron, Pearl, and Michigan. In the Burbank group, the varieties have a fairly even cortex, except the Burbank seedling,<sup>4</sup> which shows great irregularities. The latter variety differs further by having a broader cortex than the other members of the group. The Green Mountain and Rural types have as a rule a very regular cortex, though in the Green Mountain the width may sometimes vary. Climatic conditions appear to influence the development of the cortex, inasmuch as rapid growth in the early development of the tuber tends toward the formation of a broader cortex. However, it is safe to assume that a wide or narrow cortex, if constant in a number of specimens, is characteristic of the variety regardless of the external conditions under which the tuber was grown.

STONE CELLS.—When tubers of certain varieties are sectioned through the region of the bud, large lignified cells are noticed. The occurrence of these so-called stone cells is so constant that potato varieties can be divided into two classes—those in which stone cells are present and those in which they are wanting. While the presence of stone cells is a varietal characteristic, there is a great deal of variation in their number. The Rural group is distinguished by developing stone cells very sparingly, and in some varieties, like Heavy Weight, they seem to be absent altogether.

## CLASSIFICATION OF VARIETIES

Stone cells present.		Stone cells wanting.
Numerous.	Sparingly developed.	
Peachblow. Burbank (seedling). Superba Irish. Triumph. Russet Burbank. Rural.	Rural No. 1. Carman I. Green Mountain. Triumph. Early Eureka. Pearl. Charles Downing. Carman III. Gold Coin.	Rose. Heavy Weight. Ohio. McCormick. Burbank. Hebron. Russet Rural. White Rose. Spaulding Rose. Russet Burbank. Cobbler.

<sup>4</sup> Name under which William Stuart is carrying one of the Burbank strains.

**STARCH.**—The quality of starch is determined by the percentage of large grains it contains. The ability to produce a large percentage of superior grains is, according to Saare (16), a variety character, which is reasonably constant. In the breeding of varieties for a definite purpose a knowledge of the starch content of the different varieties might be an important factor which would enable the experimenter to make such crosses as would be most promising in results. Parow (12) found in his studies that in the variety Tannenberg the percentage of superior grains was as high as 50, while Weddingen yielded only 11 per cent of high-quality grains. In American varieties a group classification based on the quality of the starch grains could also be easily worked out, especially if there exist such striking differences as in Green Mountain and Cobbler as seen in Plate 2, B, C. Since material for investigation is not available at present, the information to be gained from such a study must be reserved for a future publication.

#### THE PERIDERM

The periderm of the potato has been the object of frequent investigation, and pictures of its structure may be found in any of the textbooks on botany. A comparative study of the periderm of different varieties, however, began only when differences in the disease resistance of certain varieties focused the attention of the pathologist on this natural and effective barrier to infection—the potato skin.

More than 50 years ago Sorauer made a study of the structure of the periderm of nearly 75 varieties. He made exact measurements of the thickness of the periderm, the number of cell rows, and the size of the individual cells. He noticed that deep planting tended to produce a thinner skin than shallow culture and that fertilization of the soil had the same effect. In his infection experiments with late blight he found that fertilization and deep planting produced a higher per cent of diseased plants and that the red varieties were more susceptible than the white ones. From this he concluded that thickness of the skin is correlated with disease resistance. A few years later, however, it was shown by Ress and Bretschneider that the very thick-skinned varieties were even more readily attacked than the thin-skinned ones, and that thickness of the skin in itself did not insure immunity. In 1908 Kreitz (7) extended the work of Sorauer by making extensive investigations on the effect of environmental and soil factors on the structure of the periderm. Kreitz found that the thickness of the periderm of a given variety was not a constant factor, but varied with changes in environmental conditions. In opposition to Sorauer he noticed that dryness tended to produce a thin skin and that the different varieties behaved variously. Thickness of the periderm acquired in a new locality would be retained for a generation or more, even after the tubers were transferred back to their original home. Finally, however, they showed the same kind of periderm as they originally possessed. Application of fertilizers gave varying results in that potassium and nitrogenous fertilizers tended to produce a thin skin, phosphoric acid fertilizers a comparatively thick skin. Since the thickness of the periderm in itself appears to be no factor in disease resistance, Kreitz suggested that the ability to regenerate periderm cells in wounded places would be of importance in preventing infection with bacteria. In this connection the work of Appel (1) is of significance. Appel found that in the variety

Daber, which is resistant to bacterial wilt, new cork cells appeared 6 hours after injury, while in the thin-skinned and susceptible variety Apollo, new division walls in the periderm formed only after 36-48 hours had elapsed. Lutman (8) emphasizes the fact that it is almost impossible to eliminate the personal equation in determining the thickness of the periderm, since on the same tuber thin and thick areas are commonly found. He noticed that application of fertilizers does not affect the thickness of the skin and that varieties resistant to scab have a thick periderm associated with a type of lenticel which is close textured and partly buried under the skin.

In the present study of the structure of the periderm similar variability which could not be correlated with the variety or the external conditions of growth was encountered. This must be borne in mind in connection with the following descriptions of the periderm, as it appeared to be most typical in the varieties studied.

#### STRUCTURE OF THE PERIDERM IN POTATO VARIETIES GROUPED ACCORDING TO STUART (19)

**COBBLER.**—Periderm normally thick with somewhat rough or scaly surface. Layer measures 130-156 microns and is composed of 8-9 rows of cells. Variation in thickness is not infrequent, as can be seen in Plate 10, A, B, and Figure 7. The cells vary greatly in size and arrangement. The radial rows are interlocked and the cells narrow, which gives the periderm a compact texture. The average size of most cells is 78 by 13.5 microns. The transition zone is rather wide and gradual (4-5 rows). The cells are elongated, rectangular, or pointed.

**TRIUMPH.**—Periderm of medium thickness, covered by a thin, rough crust. Layer measures 130 microns and is composed of 6-7 rows of cells. Occasionally as many as 10 rows can be counted, the total width of the layer reaching 156 microns or even more. The periderm cells show a fairly regular arrangement in radial rows; the width of the rows, however, varies considerably. The average cell dimensions are 82 by 13-15.6 microns. The transition zone is wide and the change in cell type is gradual. All transition cells are usually narrow and elongated, but near the cortex they become oblong or even cubical. (Pl. 10 and fig. 8.)

**MICHIGAN.**—Periderm of medium thickness; surface usually smooth, but in Early Eureka rough and scaly. Thickness of periderm layer 130 microns; average number of rows about 9. The cells are rather uniform and show a distinct arrangement in unbroken radial rows. The average length of the cells is 85 microns. In Early Eureka the cells are wider, measuring on the average 104 microns. The transition to the cortex is gradual. (Pl. 10, D, and fig. 7.)

**ROSE.**—Periderm of medium thickness and smooth. Layer measures 135 microns and is composed of 6-8 rows of cells. The cells are arranged in even radial rows. The width of the rows varies. There are commonly two types of cells—wide ones, measuring 82 by 13-15.6 microns, and very narrow ones. There is only a narrow transition zone, but transition is nevertheless gradual because the transition cells greatly resemble the periderm cells. (Pl. 10, E, F, and fig. 7.)

**EARLY OHIO.**—Periderm of medium thickness and covered with a thin crust. Layer measures 117-130 microns and is composed of 6-8 rows. The cells are arranged in even radial rows of elongated, mostly narrow cells. The two types of cells measure 78 by 15.6 and 38 by 14.8-15.6 microns, respectively. There is a broad transition zone; transition itself

is very gradual. The transition cells are oblong or pointed (Pl. 10, G, and fig. 8).

HEBRON.—Periderm normally thick, smooth, or covered with a thin rough crust. Layer measures 143 microns and is composed of 8-9 rows of cells. The rows are usually broad, but the uniformity is broken in

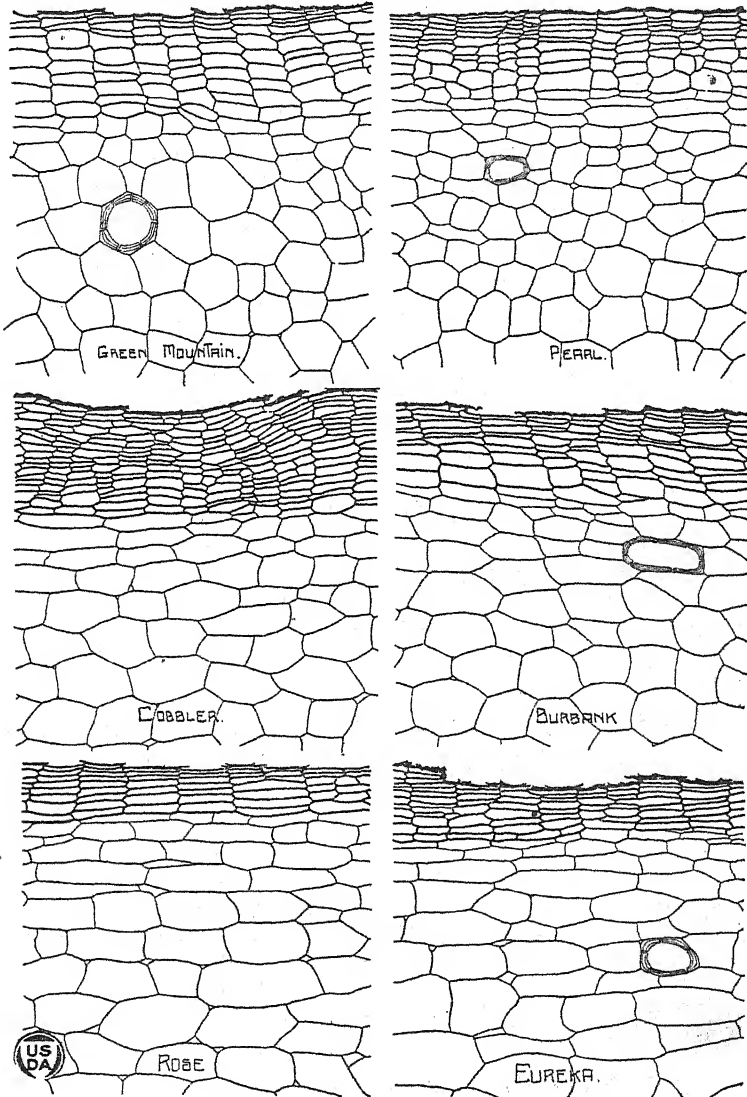


FIG. 7.—Semidiagrammatic drawings of periderm of potato tubers.  $\times 40$ .

that narrow rows are often interpolated between wide rows. The larger cells measure, 137 by 14.8 microns, on the average. A narrow but gradual transition zone connects the periderm with the cortex. The cells of the transition zone are rectangular; as they approach the cortex they become oblong or polyhedral (Pl. 10, H, and fig. 8).

BURBANK.—Periderm very wide and covered with a rough and jagged crust in the Russet types. Thickness of periderm layer is 156–169 microns; average number of rows 7–11. The cells show a fairly regular arrangement in radial rows; the width of the rows, however, varies greatly. The most common type of cell measures 78 by 18.2 microns.

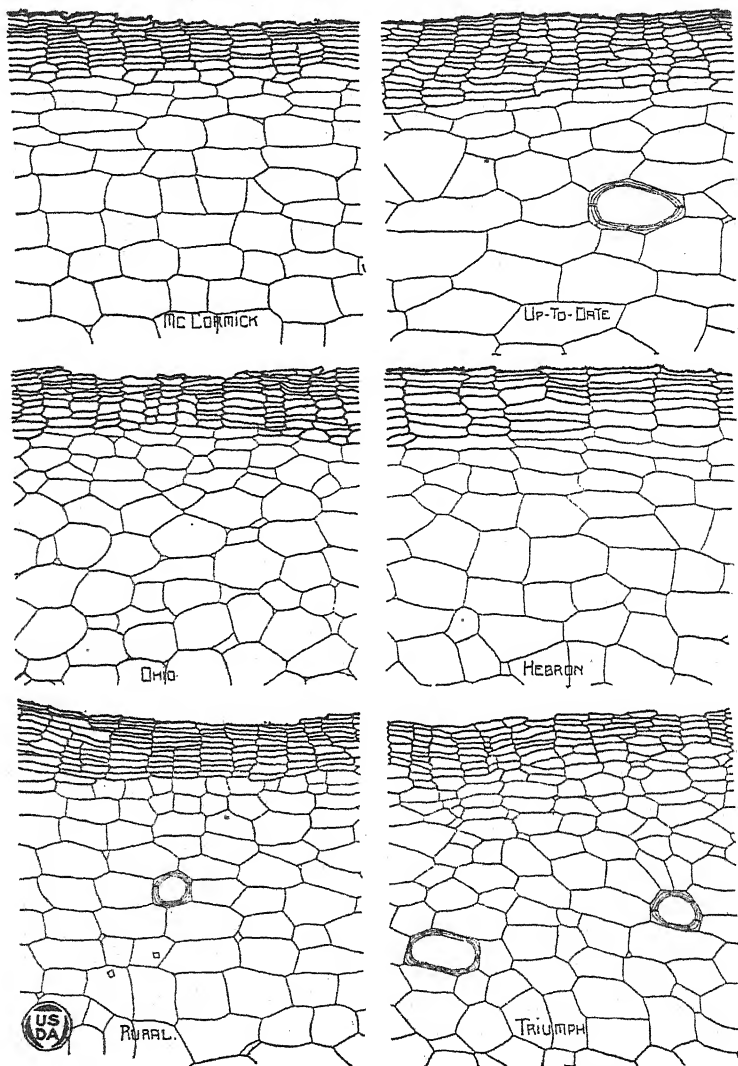


FIG. 8.—Semidiagrammatic drawings of periderm of potato tubers.  $\times 40$ .

Transition to the cortex is effected by four rows of small periderm-like cells (Pl. 10, I, J, and fig. 7).

GREEN MOUNTAIN.—Periderm thin or of medium thickness, covered with thin rough crust. Layer measures 104–125 microns, occasionally more. Number of rows seven to nine. The cells are uniform and show

distinct arrangement in radial rows. The large cells measure 104 by 14.8 microns, the narrow type 52 by 14.8 microns. Transition is gradual, but the transition cells resemble those of the cortex rather than the periderm (Pl. 10, K, L, and fig. 7).

**RURAL.**—Periderm thin or of medium thickness, usually more extensive in the Russet type. The surface is smooth or, in Russet Rural, covered with a thick, scaly crust. Layer measures 130 microns, number of rows about seven. Though sometimes uniform, the periderm is more conspicuous by irregularity of the arrangement of the rows and of the individual cells. The most common type of cell measures 91 by 18.2 microns, but cells as wide as 156 microns are not uncommon. The transition to the cortex is narrow and abrupt. The transition cells are rectangular, but sometimes elongated and narrow, thus more closely resembling those of the periderm (Pl. 10, M, N, and fig. 8).

**PEARL.**—Periderm thick and covered by a rough thick crust. Layer measures 156 microns and is composed of 8–10 rows. Arrangement of rows somewhat irregular with much variation in the width of the rows. Most cells measure 91 by 13 microns on the average. Transition zone is of medium width. The cells are elongated and irregular. Transition appears to be more abrupt than gradual (Pl. 10, O, and fig. 7).

**McCORMICK.**—Periderm thick and covered by rough crust. Layer measures 156 microns and is nine rows wide. The cells show a regular arrangement in radial rows of fairly even width. In general the periderm cells are narrow, measuring only 52 by 18.2 microns. Transition is very abrupt; often only a single transition row is present. In the Peachblow several rows of somewhat elongated cells are commonly found (Pl. 10, P, Q, and fig. 8).

**UP-TO-DATE.**—Periderm of varying width, smooth or covered by thin crust. Layer measures from 104 to 160 microns and is composed of 7–14 rows of cells. The cells show a fairly even arrangement, but the width of the rows varies. On the whole the periderm layer has a very compact appearance. The most common type of cells measures 78 by 15.6 microns, on the average. The transition zone is wide; transition itself is gradual. The cells of the transition zone are usually much elongated (Pl. 10, R, and fig. 8).

#### SUMMARY

(1) Developmental studies on the potato tuber lead us to conclude that:

(a) The periderm is formed jointly from the epidermis and the hypodermis. Continuity of the periderm is assured by the development of a phellogen arising in the hypodermis.

(b) The cortex of the tuber forms a very narrow band of tissue between the periderm and the outer circle of phloem groups. The cells contain the pigment in case of colored varieties; protein crystals, tannins, and a small amount of starch.

(c) The pith forms the narrow central core of the tuber, but is continuous with the eyes by means of lateral branches. The cells of the pith are poor in starch and have a higher water content than the rest of the tuber issue.

(d) The vascular tissue: The vascular ring as it appears to the naked eye constitutes a narrow band of tissue which contains the xylem and the secondary phloem. The broad bands of storage parenchyma in

which numerous groups of phloem are embedded, though procambial in origin, appears distinct from the vascular ring and is not the result of cambial growth.

(2) The morphological advantage which the apical eye possesses over the basal ones seems to offer the most reasonable explanation for the dominance of the former.

(3) The nucleus of the sieve tubes disappears before the elements fully matured. The peculiar protozoan-like structures observed in have young sieve tubes are of cytoplasmic origin and have only a temporary existence.

(4) In a consideration of the chemical constitution of the tuber, the protein crystals, the tannin vesicles, and the solanin are of special interest. The protein crystals occur abundantly in the peripheral cells of the cortex; the tannin vesicles in the region of the buds, especially at the time of sprouting; the solanin accumulates in large quantities in the region of the buds, where it appears to be of importance in the metabolism of the growing plant. If germination is held back, solanin may accumulate in abnormal quantities.

(5) The phloem of the stolon occupies about 24 per cent of the area of the stolon cross section. Its extensive development, in response to the apparent need for increased food movement, strengthens the view that the phloem is, after all, the most important channel for the translocation of organic substances.

(6) A study of such internal characters as might aid in the task of grouping potato varieties or establishing their relationship indicates that the presence or absence of stone cells is the only definite character which can be used successfully in a classification scheme.

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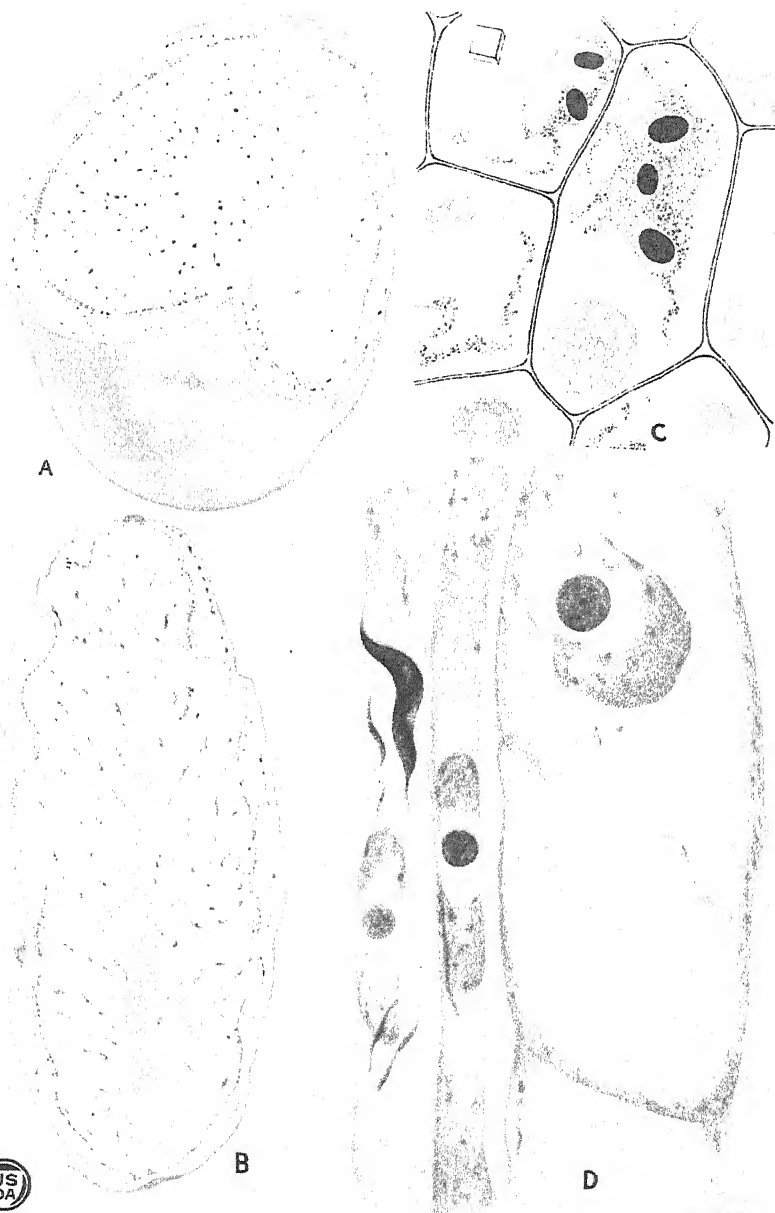
# PLATE I

A.—Potato tuber cut to show transverse and tangential surfaces.

B.—Radial section of tuber to show pith connections to bud. The red line indicates the location of the xylem part of the vascular tissue. Notice that the xylem is confined to a narrow ring, while the phloem is scattered throughout the tuber tissue.

C.—Cortical cells from region just below a bud. The cells contain starch (violet), protein crystals (green) and tannin vesicles (orange yellow).

D.—Protein bodies in sieve tube of young stolon. The cell to the left is a young sieve tube containing a nucleus and spiral bodies (spiral bodies and nucleolus colored red). Adjacent to the sieve tube is a companion cell and next to it a large parenchyma cell. Drawing is a reproduction of a preparation stained with methyl green and acid fuchsin according to Altmann.



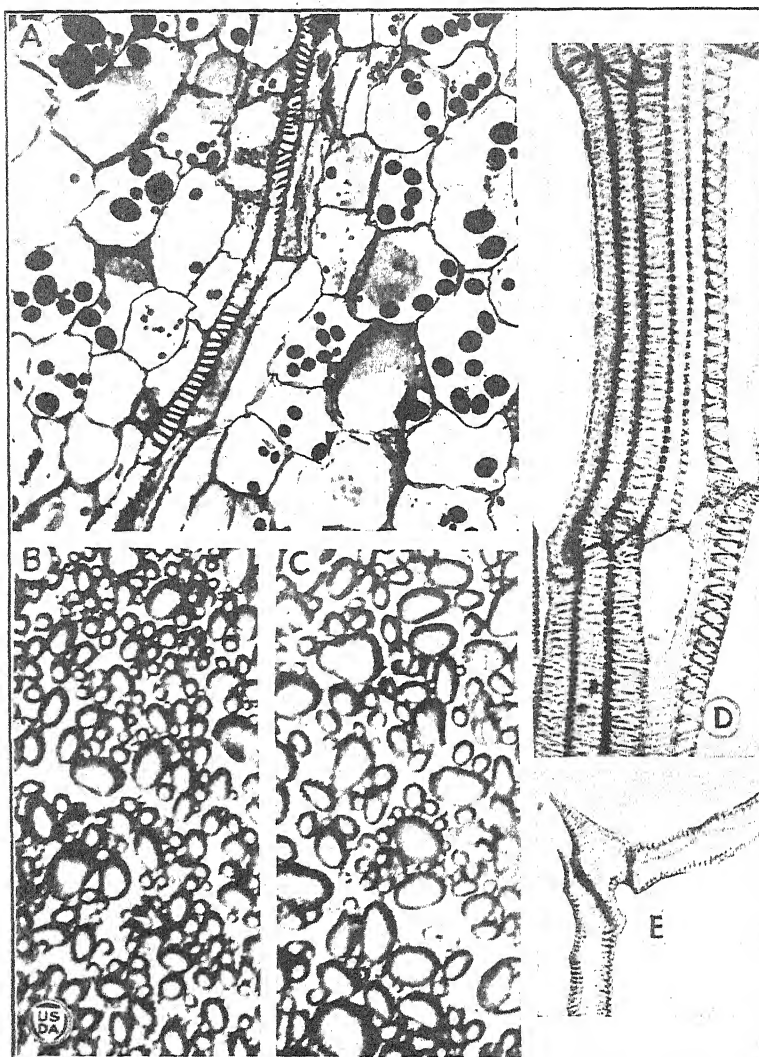
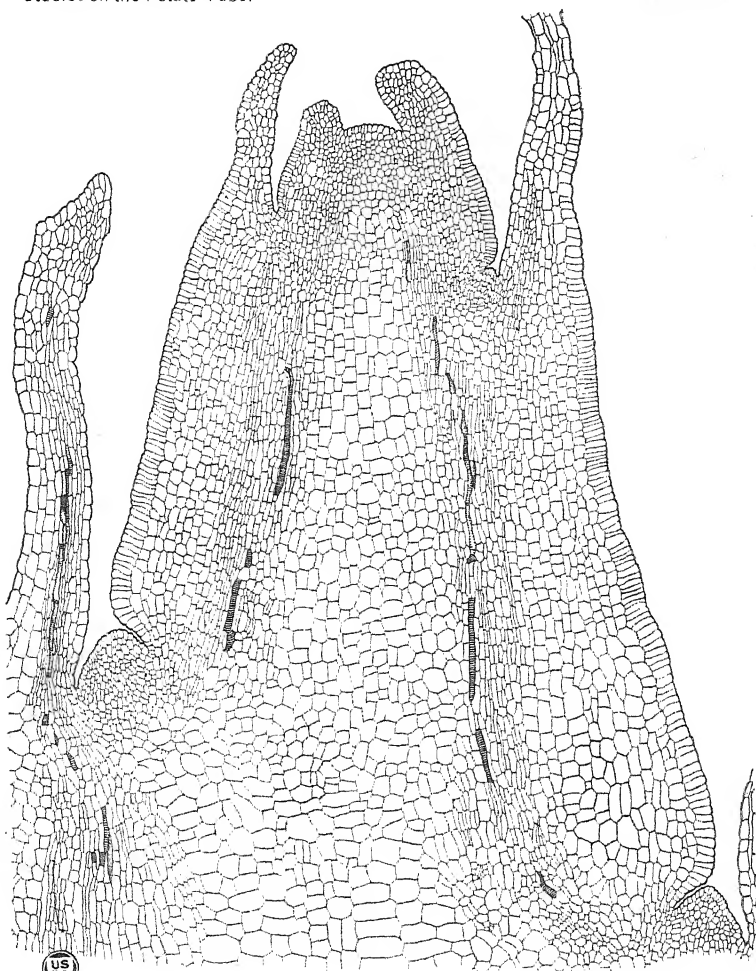


PLATE 2

- A.—Longitudinal section through vascular ring of mature tuber.  $\times 129$ .
- B.—Starch grains from the variety Irish Cobbler.  $\times 125$ .
- C.—Starch grains from the variety Green Mountain.  $\times 125$ .
- D.—Protoxylem elements from mature tuber.  $\times 125$ .
- E.—Secondary xylem vessels from mature tuber.  $\times 125$ .

PLATE 3

Longitudinal section of stolon tip prior to tuber formation.  $\times 130$ . (Drawing is a copy of photomicrograph.)



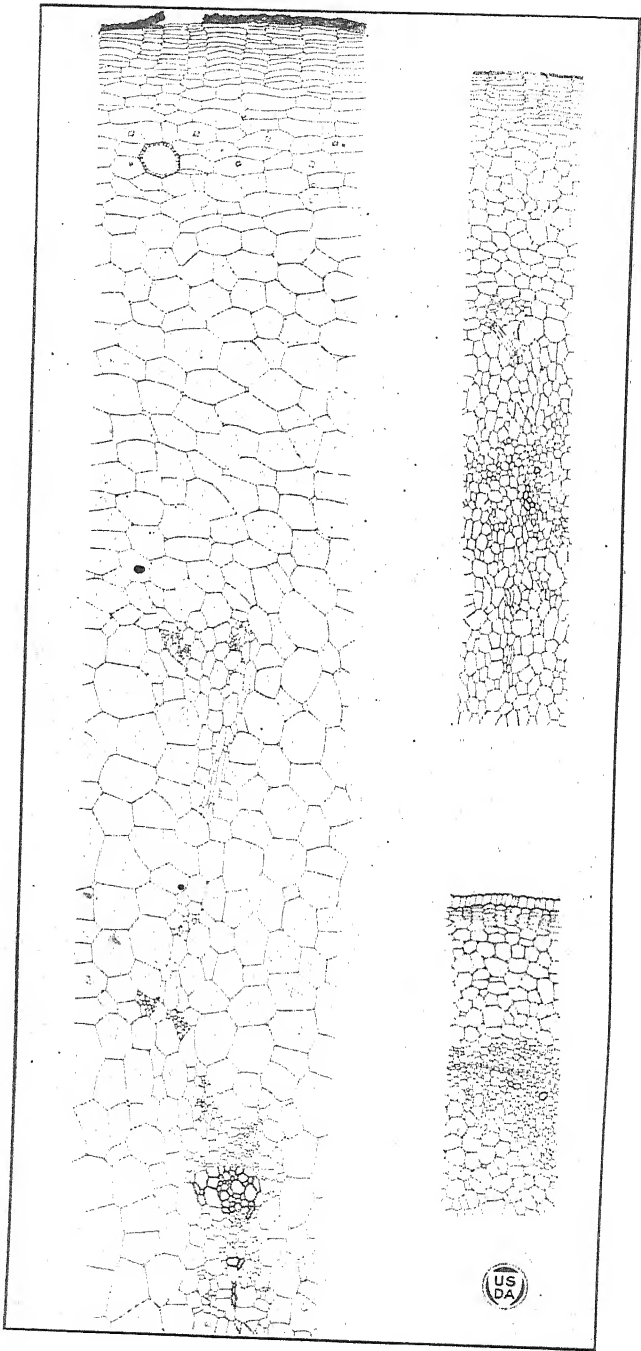




PLATE 4

Stages in the development of the tuber.

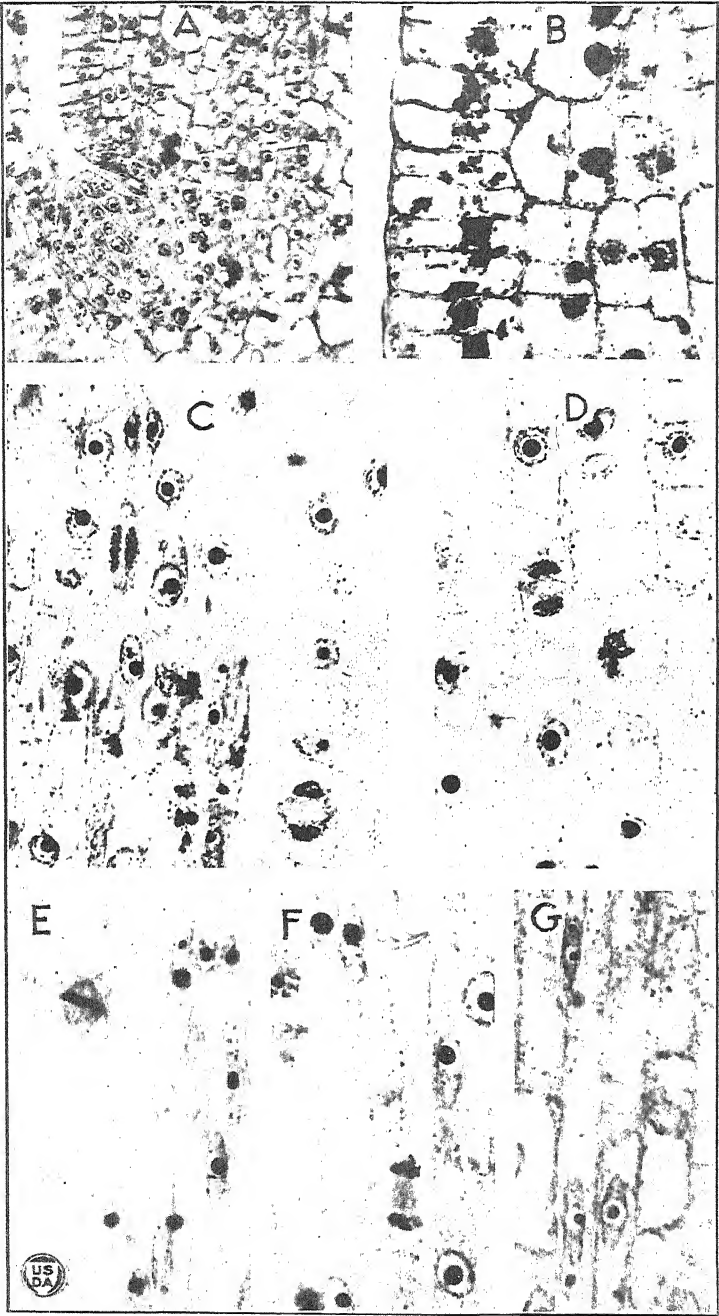
A.—Section through periderm, cortex, pericyclic region and vascular ring of mature tuber.  $\times 45$ .

B.—The same as in A, except that tuber is only 10 mm. in diameter.  $\times 45$ .

C.—The same as A and B, but tuber is only 2.5 mm. in diameter.

PLATE 5

- A.—Section through tuber bud initial.  $\times 113$ .
- B.—Radial section of epidermis and adjacent cortical cells of stolon tip.  $\times 583$ .
- C.—Mitotic division in young stolon cells.  $\times 583$ .
- D.—Mitotic division—late meta- and telo-phase.  $\times 583$ .
- E.—Mitotic division—metaphase.  $\times 583$ .
- F.—Mitotic division—anaphase.  $\times 583$ .
- G.—Phloem cells with elongated nuclei.  $\times 583$ .



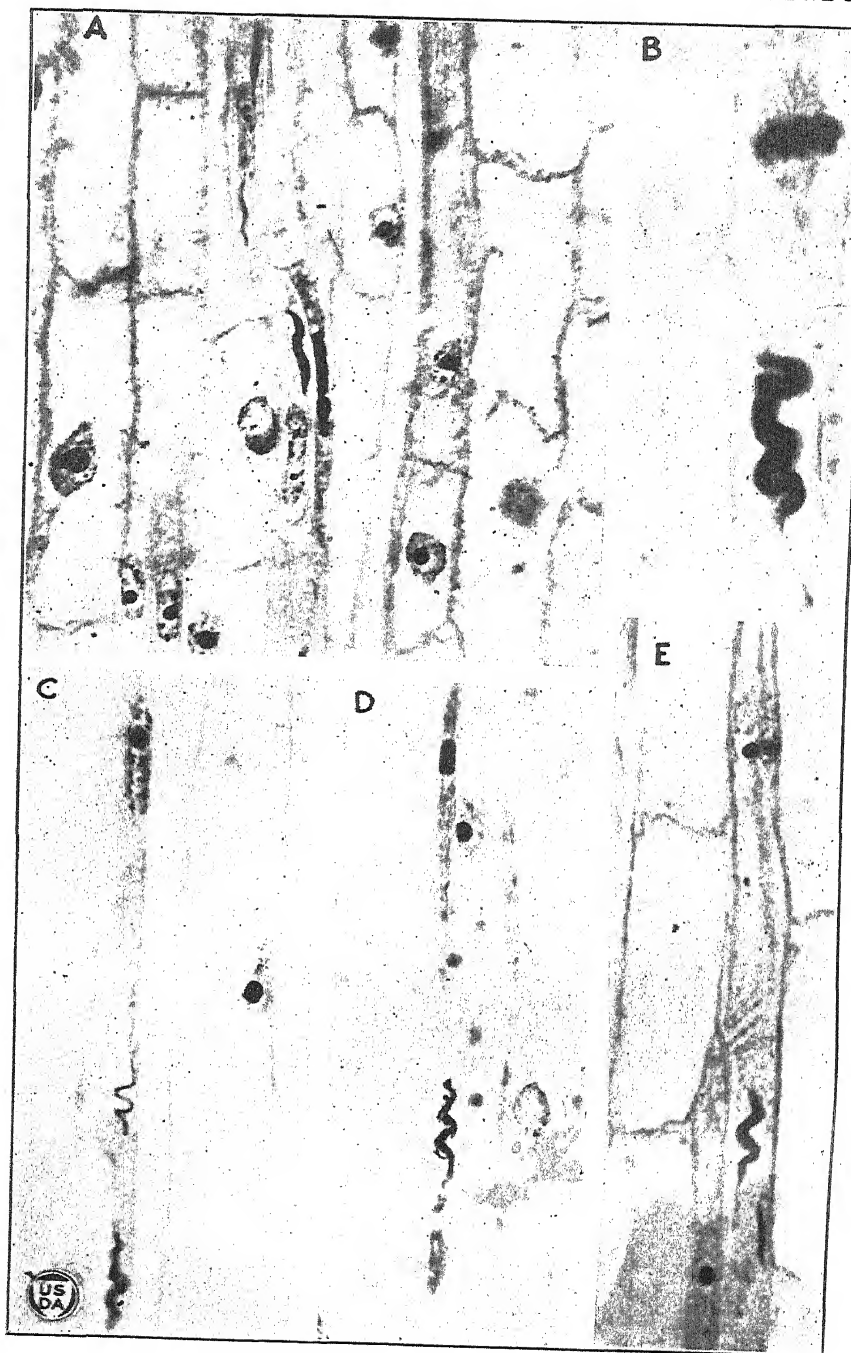
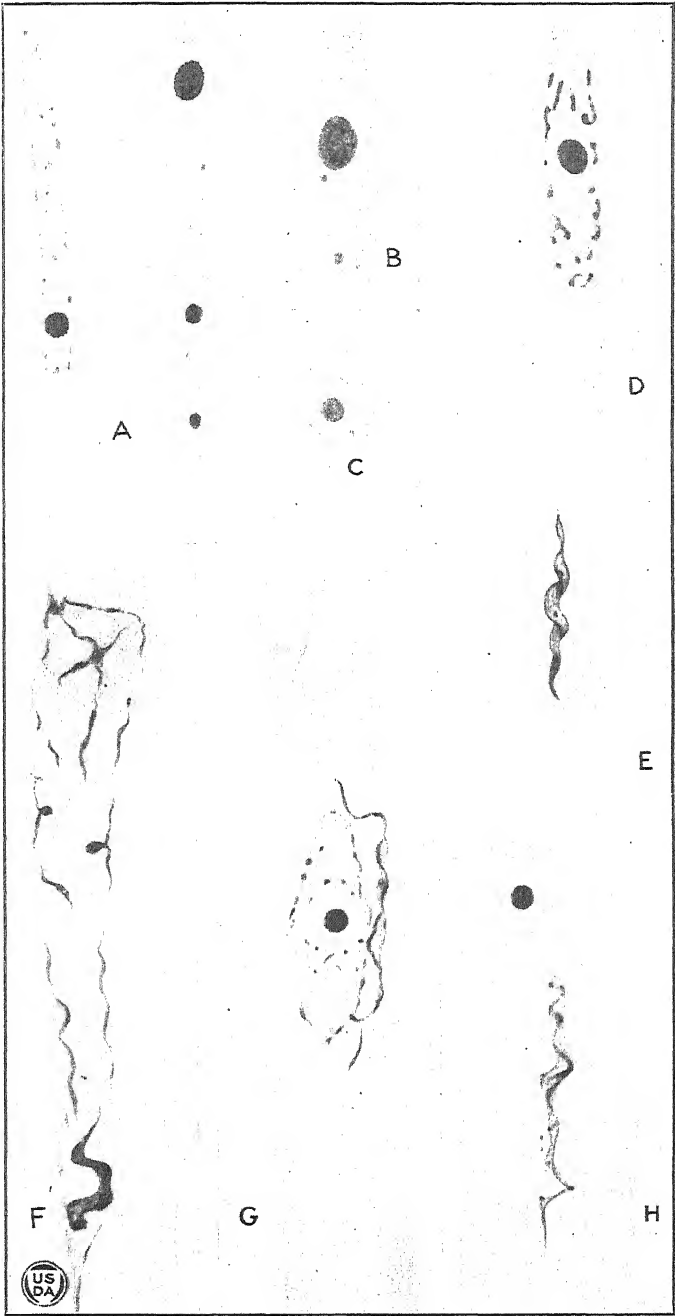


PLATE 6

- A.—Sieve tube with nucleus and slime body.  $\times 800$ .
- B.—Sieve tube with slime body and nucleus in metaphase.  $\times 1500$ .
- C.—Two sieve tubes with large nucleus and two slime bodies.  $\times 800$ .
- D.—Sieve tube with slime body. Notice that the slime body is not homogeneous throughout.  $\times 800$ .
- E.—A more mature sieve tube with a large and small slime body.  $\times 800$ .

PLATE 7

- A.—Two elongated nuclei from cells of procambium.
- B.—Typical nucleus of immature vascular cell.
- C.—Typical nucleus of the apical meristem.
- D.—Elongated nucleus in prophase.
- E.—Corkscrewlike body of young sieve tube.
- F.—Large aggregate of sieve-tube bodies.
- G.—Immature sieve tube with nucleus and sieve-tube body.
- H.—Sieve-tube body showing differential staining reaction. Body is in appearance and structure not unlike certain Protozoa.



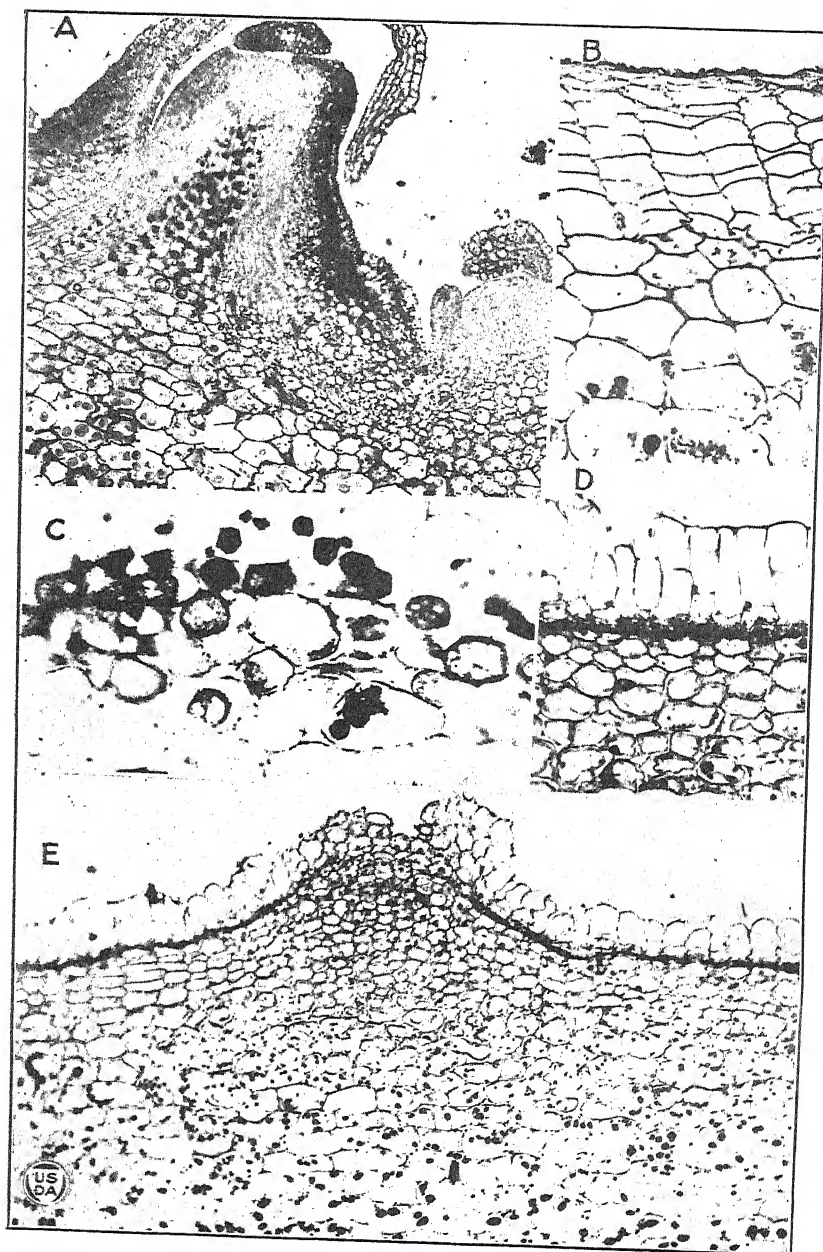


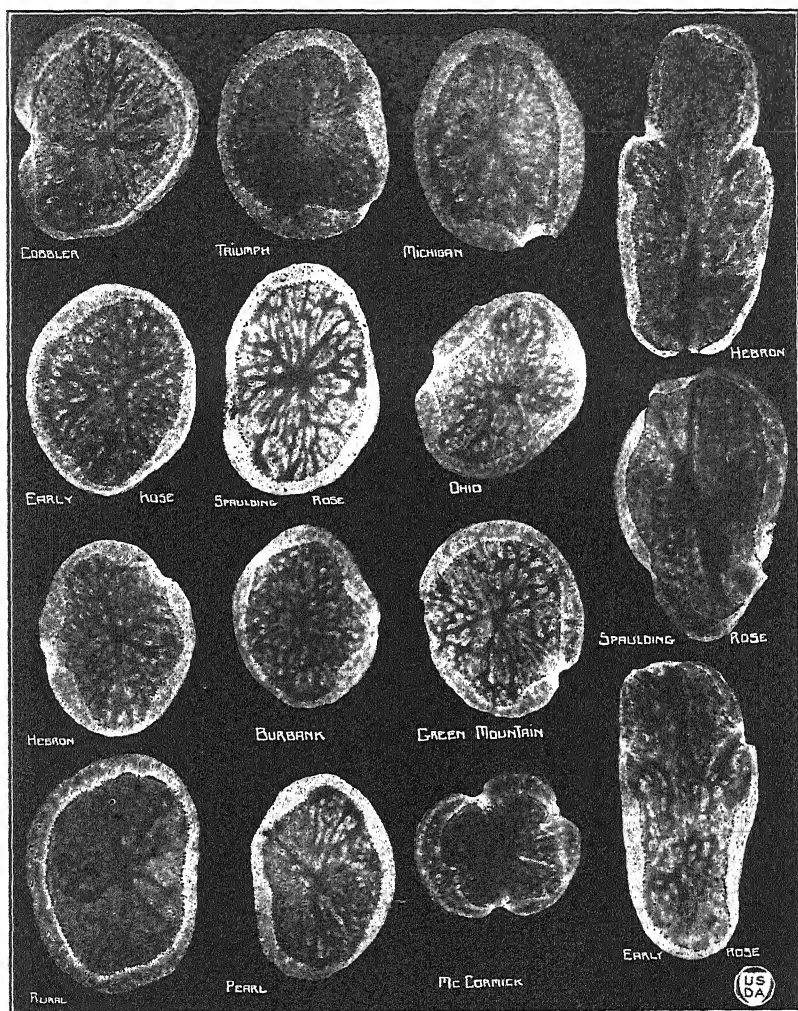


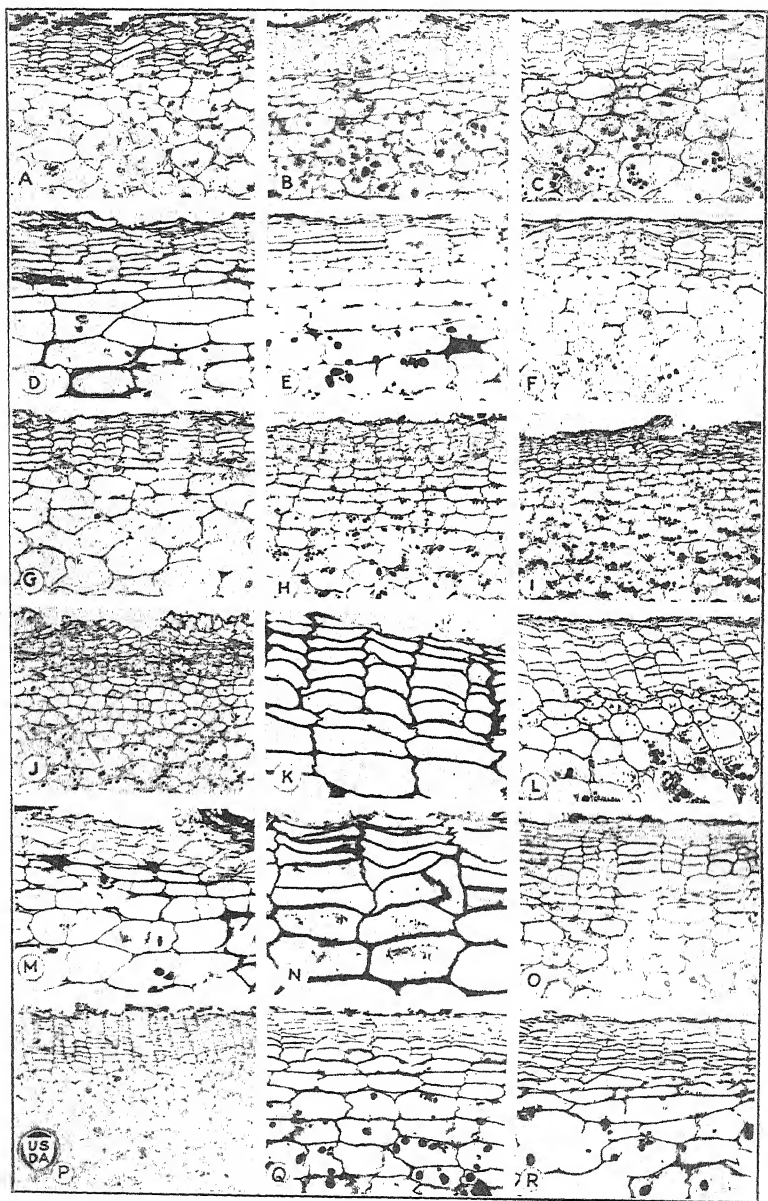
PLATE 8

- A.—Tannin vesicles in parenchyma cells of bud.  $\times 74$ .
- B.—Cross section of a normal potato periderm.  $\times 133$ .
- C.—Tannin vesicles in cells of cortex, showing inner structure.  $\times 312$ .
- D.—Cross section of a hypertrophied periderm.  $\times 133$ .
- E.—Cross section of lenticel with hypertrophied periderm.  $\times 133$ .

PLATE 9

Cross and longitudinal sections of mature tubers.  $\times \frac{13}{7\frac{1}{2}}$ .





# PLATE 10

Cross sections through the periderm of tubers.

A.—Irish Cobbler.....	×	36
B.—Irish Cobbler.....	×	36
C.—Bliss Triumph.....	×	36
D.—Michigan.....	×	36
E.—Rose.....	×	36
F.—Rose.....	×	36
G.—Ohio.....	×	36
H.—Hebron.....	×	36
I.—Burbank.....	×	36
J.—Burbank.....	×	36
K.—Green Mountain.....	×	155
L.—Green Mountain.....	×	36
M.—Rural.....	×	36
N.—Rural.....	×	155
O.—Pearl.....	×	36
P.—McCormick.....	×	36
Q.—McCormick.....	×	36
R.—Up-to-date.....	×	36



# OCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON OTHER HOSTS<sup>1</sup>

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## INTRODUCTION

In the practical control of a plant disease caused by a fungus it is obviously of first importance to know whether the causal organism is confined to a particular host or grows, either as a parasite or saprophyte, on other hosts in the same locality. In the present paper evidence is presented to show that the cane blight fungus, an active parasite on the cultivated currant, occurs also on at least two unrelated hosts.

## CURRANT CANE BLIGHT

The disease of cultivated currants, now generally known as cane blight, was first reported more than 30 years ago by Fairchild<sup>2</sup> who found it in the Hudson Valley about Highland and Milton, N. Y. Of subsequent investigations, the most important is that made by Grossenbacher and Duggar<sup>3</sup> in the same region during the period from 1907 to 1911. One of the writers has had this disease under observation since 1917 and has made culture and inoculation experiments in various parts of the eastern United States.

The most conspicuous symptom of this disease and the one from which its common name is derived is the wilting of the leaves and fruit of infected plants. This apparently sudden wilting occurs most frequently during the period when the fruit is being matured and is usually caused by the girdling of the main stem by the fungus which has grown in from a lateral branch.

Infection generally occurs through a terminal or lateral bud and the parasite develops basipetally, invading all woody structures. Young shoots which have become infected usually die at the distal ends during the same season. The growth of the fungus apparently continues throughout the growing season during which large branches or even main stems may be blighted at any time. However, as stated above, the wilting is usually most conspicuous just before the fruit ripens, perhaps because of the greater demands on the moisture supply at that time. The disease may be recognized with a fair degree of certainty by the peculiar blackened appearance it gives to the wood and pith. So far as has been determined, the roots are not infected.

The disease is known to occur in Massachusetts, Connecticut, New York, Ohio, Pennsylvania, New Jersey, Maryland, and Virginia. Currants are grown only locally in Maryland and Virginia. The disease is very serious in New Jersey and has been an important factor in the reduction of the currant acreage in that State during recent years. It is

<sup>1</sup> Received for publication Jan. 27, 1924.

<sup>2</sup> FAIRCHILD, D. G. NOTES ON A NEW AND DESTRUCTIVE DISEASE OF CURRANT CANES. (Abstract) Bot. Gaz. 16: 262. 1891.

<sup>3</sup> GROSSENBACHER, J. G., and DUGGAR, B. M. A CONTRIBUTION TO THE LIFE-HISTORY, PARASITISM, AND BIOLOGY OF BOTRYOSPHERA RIBIS. N. Y. State Agr. Exp. Sta. Tech. Bul. 18: 115-190, illus. 1911.

destructive also, especially during certain seasons, in the Hudson Valley of New York and in southern Connecticut. Farther north, however, it seems to be of much less importance, and has not yet been found to be serious in northern New England or in Canada. The fungus has not been found in Alaska, though currants are commonly grown there.

No effective method of control for currant cane blight has been developed. Systematic cutting out and burning of the diseased parts has, however, served to keep the disease down to a point where currants are commercially profitable even though the disease is commonly present.

#### THE CAUSAL ORGANISM

Grossenbacher and Duggar showed by inoculation experiments that currant cane blight was caused by a fungus to which they gave the name *Botryosphaeria ribis*. They also called attention to the frequent occurrence on currant canes of a morphologically similar fungus which is not parasitic and which they designated as *Botryosphaeria ribis* forma *achromogena*. This form name was chosen because the saprophyte lacks the most conspicuous cultural character of the parasite, namely, the production on starch paste or other starchy media of a bright pink color during the first three or four days of growth.

The observations of the senior writer as to the causal organism have fully confirmed those just reviewed. Currant cane blight seems to be everywhere associated with the chromogenic *Botryosphaeria*, and infection with the chromogen is readily secured from inoculations made at the right time.<sup>4</sup> On the other hand, the results of inoculations with the non-chromogenic form have been uniformly negative. Grossenbacher and Duggar consider the two forms morphologically practically identical, the ascospores measuring 16–23 $\mu$  by 5–7 $\mu$  and the pycnosporos 16–31 $\mu$  by 4.5–8 $\mu$ . The material examined by the writers varies through somewhat wider limits than those given by Grossenbacher and Duggar, perhaps due to the fact that it has been derived from a wider geographical range. Comparison of our material, however, both with the authentic material distributed in Bartholomew's *Fungi Columbiani*, Numbers 3408 and 3409, and with the original slides of Grossenbacher and Duggar now preserved at the Geneva Experiment Station, as well as culture and inoculation work, leaves no doubt that the fungus described by them is identical with that used in our inoculation work on currant.

#### OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON HORSE-CHESTNUT

Our work on the currant cane blight fungus forms a part of the extensive studies of the fungi belonging to this group which have been carried on for more than 15 years by Shear and his associates.<sup>5</sup> During this period, as stated by Shear and Beckwith,<sup>6</sup> fungi which closely resembled *Botryosphaeria ribis* morphologically have been secured from several hosts. However, although one strain collected on *Viburnum* showed chromogenesis when cultured on starchy media, no direct evidence of the occurrence of the currant cane blight fungus on hosts other than *Ribes* sp. was secured until 1921.

<sup>4</sup> GROSSENBACHER, J. G., and DUGGAR, B. M. OP. CIT., p. 137.

<sup>5</sup> SHEAR, C. L. LIFE HISTORY OF MELANOPS QUERCUM (SCHW.) REHM FORMA VITIS SACC. (Abstract.) Science 31: 748. 1910.

<sup>6</sup> SHEAR, C. L., and BECKWITH, A. M. LIFE HISTORIES OF MELANOPS. (Abstract.) Phytopathology 6: 109. 1916.



In the fall of that year one of the writers, during a collecting trip through the currant-growing regions of New York, picked from a horse-chestnut tree (*Aesculus hippocastanum*) growing on a lawn between Milton and Marlborough, a dozen or more mature, apparently healthy fruits. Not more than 20 feet from the tree was a good sized currant patch in which cane blight was abundant. The fruits were collected, however, with the expectation that a nonchromogenic fungus of this type would develop, as one had been repeatedly secured on several successive years from *Aesculus* fruits collected in the District of Columbia. The fruits from New York were taken to Washington and placed in a moist chamber for several weeks, when they became covered with the pycnidia typical of *Botryosphaeria ribis*. Single spore cultures made from this material showed, however, the chromogenesis hitherto associated only with the currant parasite. Repeated comparative cultures were made and the horse-chestnut fungus proved to be indistinguishable from the known parasite from currant.

Late in December of that year 24 healthy 3-year-old currants of the Wilder and Red Dutch varieties were secured from northern New York and planted in a greenhouse at Arlington Experiment Farm, Rosslyn, Va. When the plants had reached the stage most favorable for infection,<sup>7</sup> one-third of them, about 30 in all, were inoculated with the horse-chestnut fungus, an equal number with the parasite from currant, and the remainder were maintained as controls, that is, were wounded with a sterile knife but not inoculated.

The results were conclusive. Nineteen of the 30 inoculations with the fungus from horse-chestnut developed the typical cane blight and were dead by the middle of May. Only 16 of the twigs inoculated with the fungus from currant developed the disease, but this difference is no doubt accidental, as the percentage of infection is as high as that usually secured in our previous work out of doors. None of the control plants became diseased. Single spore cultures from the pycnosporangia which matured later on the dead limbs showed that the chromogenic fungus was present in every case. All the plants which had been inoculated with the horse-chestnut fungus were transplanted during the latter part of June to a shaded place isolated from any other currants.

#### OCCURRENCE OF THE CURRANT CANE BLIGHT FUNGUS ON ROSE

In October, 1922, diseased canes from a plant of *Rosa setipoda* growing in the rose garden at the Bell Station, Maryland, were referred by the Federal Horticultural Board to one of the writers for identification of the fungus present. Microscopical examination showed that pycnidia of the *Dothiorella* type were fruiting on the stems, which were severely cankered. In cultures on corn meal chromogenesis similar to that associated with the cane blight fungus was evident after about 24 hours. Single pycnosporangia cultures were likewise chromogenic. This was the only observation of the disease in 1922, but late in March, 1923, a few discolored areas were found on the canes of three large plants, one of which was the *Rosa setipoda* plant from which the first collection was made. Pycnidia, bearing pycnosporangia similar to those present on the October material, were distributed over the lesions and from these the chromogen was again isolated.

<sup>7</sup> GROSSENBACKER, J. G., and DUGGAR, B. M. OP. CIT., p. 137.

The cultures from the fungus on rose were indistinguishable from those of the cane blight fungus and the pycnospores were also in agreement with those of *Botryosphaeria ribis*. Therefore it seemed very likely that we were again dealing with the currant blight fungus on another unrelated host. Moreover, on the rose the fungus was apparently parasitic. Accordingly, it was planned to conduct inoculation experiments with roses as well as currants.

The inoculation experiments on currant were conducted as described above for the material from horse-chestnut, except that 2-year-old currant bushes were used, and no parallel inoculations were made with the fungus from currant. Cultures made from a single pycnospore were used. More than half the twigs inoculated with the rose fungus developed typical cane blight and reisolations showed the presence of the chromogenic fungus in every case. The controls remained healthy.

#### PARASITISM OF CURRANT CANE BLIGHT FUNGUS ON ROSE

For the inoculations on the rose, 12 potted plants of the variety Columbia (Hybrid Tea) which had been propagated from cuttings were used. On April 2, 1923, 4 of the plants were inoculated with subcultures from those used in the currant inoculations, 4 with cultures made from the specimens collected in March, while the remaining 4 plants were kept for controls. The method of making the inoculations was the same as for currant. About two weeks after the inoculations were made all of the inoculated plants showed infection and the imperfect stage of the *Botryosphaeria* was fruiting on some of the stems. A week later the foliage above the point of inoculation had become yellowish green and was beginning to wilt, and by the last of the month it was dead and dry (Pl. 2). The controls remained healthy and the wounds which had been made in the stems by the sterile scalpel had healed. The perfect stage of the *Botryosphaeria* was observed on a number of the infected plants two months after the plants were inoculated. Cultures made from the ascospores, as well as the earlier reisolations from infected stems, were chromogenic and produced the imperfect stage of the fungus in about three weeks. Thus the pathogenicity of the fungus on the rose was established.

When the rose garden was again visited late in June a number of plants, including the three on which the cankers were present in March, were found to be severely infected. Many canes 6 or 7 feet in height were killed to the ground, the brown, withered foliage being still attached. The pycnidial pustules of the *Botryosphaeria* were fruiting abundantly on these blighted stems and on one of the specimens brought to the laboratory immature perithecia were present.

On October 25 another survey of the garden was made and the disease was observed on still other varieties. The perfect stage of the fungus was fruiting abundantly on many of the specimens taken at this time and single ascospore isolations developed the typical chromogen. On this date the *Botryosphaeria* was collected for the first time on an American species, *Rosa pratincola*. Otherwise the disease has been observed only on rose species from China or their hybrids, as follows: *R. adenosephala*, *R. banksiopsis* ×, *R. bella* ×, *R. bella* × *moyesii*, *R. caudata*, *R. heritierana*, *R. moyesii*, *R. moyesii* × *canina*, *R. multibracteata* ×, *R. setipoda*, and a number of other varieties. There is nothing to indicate, however, that the fungus itself was imported from China. Cane blight occurs commonly on

currants in the near neighborhood, from which the fungus might easily have spread to the roses.

The symptoms produced by the currant cane blight pathogene on the rose differ somewhat from those on the currant. In some cases the entire cane is not blighted and the lesions remain as definite cankers on the stems (Pl. 1 A, B, E). The small pimple-like fruiting bodies of the fungus are distributed over the surface of the cankered bark which is of a characteristic "seal brown" <sup>8</sup> color. The region surrounding the lesions is often "vinaceous brown" shading to "dark Corinthian purple" next to the edge of the canker. Recently blighted canes, upon the development of the fruiting structures of the fungus, become speckled with slightly raised golden brown dots (Pl. 1, C). As the fungus develops, considerable areas may present this golden brown color which, with the natural sheen of the bark, makes the cane appear as though gilded. These surfaces later become dull, owing to the further development of the dark stromata beneath. In some cases the two shades of brown are arranged more or less concentrically as shown in Plate 1, D. The diseased canes may become so swollen that the bark is roughened and split longitudinally (Pl. 1, E).

The manner in which the pathogene gains entrance to the rose is not known but it seems very likely that infection usually occurs through a bud or young shoot from which it passes to the main stem (Pl. 1, A and B). Small lesions have been observed, however, where no buds, shoots, or visible abrasions were present. As in the currant, the fungus evidently penetrates all parts of the stem, having been isolated from both the cortex and wood in which the mycelium occurs intercellularly.

#### IDENTITY OF THE FUNGI FROM THE THREE HOSTS

Until the autumn of 1923, comparisons of the chromogenic fungi from horse-chestnut, rose, and currant were based on cultural characters, pycnospore size and their parasitism on currant. During July of that year, however, ascospores developed on the inoculated rose plants, and in September and October mature ascospores were found on the currants which had been inoculated with the fungus from rose and from horse-chestnut, respectively. A careful comparison of the material from the various hosts was now possible.

In Grossenbacher and Dugger's description of the currant cane blight fungus, the stromata are described as being 1-4 mm. in diameter, usually 2-3 mm. The perithecia measured 175-250 $\mu$  in width. The asci were 80-120 $\mu$  by 17-20 $\mu$  and the ascospores 16-23 $\mu$  by 5-7 $\mu$ . They describe the pycnidia as having about the same size as the perithecia, and the pycnospores as measuring 16-31 $\mu$  by 4.5-8 $\mu$ .

The perithecia of the fungus which developed on currant and rose from inoculations made with material from the various hosts were of practically the same size and agreed closely with those of the parasite which had developed from natural infections, although because of the relation of the perithecial wall to the surrounding tissues of the fungus, it is difficult to make accurate comparative measurements.

Measurements of over 200 ascospores of the currant cane blight fungus from various localities gave a range in size from 14-27 $\mu$  by 5-10 $\mu$ , though

<sup>8</sup> RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

the great majority of the spores were within  $16-23\mu$  by  $6-8\mu$ . From 25 to 50 ascospores were measured from each of the following sources: Rose from natural infection, rose from inoculations with fungus from rose, currant from inoculations with fungus from rose, and currant from inoculations with fungus from horse-chestnut. In all cases the spores agreed closely in size with those from currant. The extreme range was from  $15-25\mu$  by  $5-10\mu$ , but the spores fell mostly within the limits  $17-22\mu$

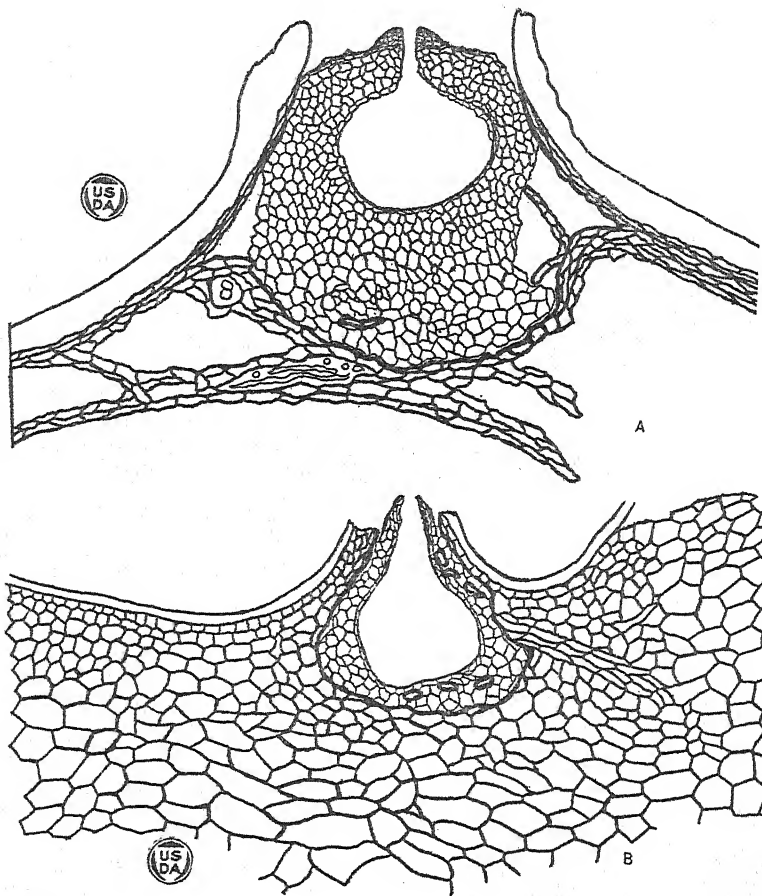


FIG. 1.—Semidiagrammatic section through single mature perithecia of currant cane blight fungus A, on currant, B, on rose. Note greater amount of stromatic tissue about perithecia which developed on currant. The host tissue is indicated by heavier lines. Both perithecia resulted from inoculations made with subcultures from the same pycnospor. X 140.

by  $6-8\mu$ . The pycnospor measurements were equally satisfactory. In about 200 spores measured, the range was  $14-31.5\mu$  by  $4-7.5\mu$ , mostly  $18-23\mu$  by  $5-7\mu$ , the pycnospor from the various hosts being in close agreement.

The size of the stromata was much more variable and is apparently directly influenced by the thickness and character of the bark within which they grow. The writers have observed on a single currant bush perithecial stromata of this fungus which varied in size from about 1 by

2-3 mm. on the smaller branches to 3-3.5 by 5-8 mm. near the base of the older canes where the bark was 2.5 mm. in thickness. Moreover, the host apparently affects to some extent the amount of stromatic tissue which develops about the perithecium. In the present study it was evident that the perithecia which matured on the inoculated rose plants had associated with them much less stromatic tissue than those which matured on inoculated currant plants (fig. 1, A, B). That the inoculum was identical in the two cases is assured by the fact that subcultures from a single ascospore culture were used in both cases. This observation alone would suggest that mere size of stroma is an unreliable character for the identification of species in this group.

#### SUMMARY

Currant cane blight is now known to occur in Massachusetts, Connecticut, New York, Ohio, Pennsylvania, New Jersey, Maryland, and Virginia, but is less severe in the northern portions of this region.

The disease is caused by a fungus to which the name *Botryosphaeria ribis* was given by Grossenbacher and Duggar, who first demonstrated its pathogenicity.

A fungus identical morphologically and in cultural characters with the currant cane blight fungus has been collected by the writers on horsechestnut and rose.

Inoculations on currants with the fungus from both these hosts have produced typical cane blight.

The fungus is apparently parasitic on several varieties of cultivated roses, and its pathogenicity has been established by inoculations on at least one variety.

The size of the stromata in this fungus is apparently directly affected by the thickness of the bark in which it develops as the stromata are smaller in thin currant bark than in thick bark, and uniformly smaller on rose than on currant.

In view of the agreement of the fungi from the different hosts in cultural characters, morphology, and parasitism, the conclusion seems amply warranted that they are identical, and that the fungus hitherto known only as the causal organism of currant cane blight does actually occur on other hosts.

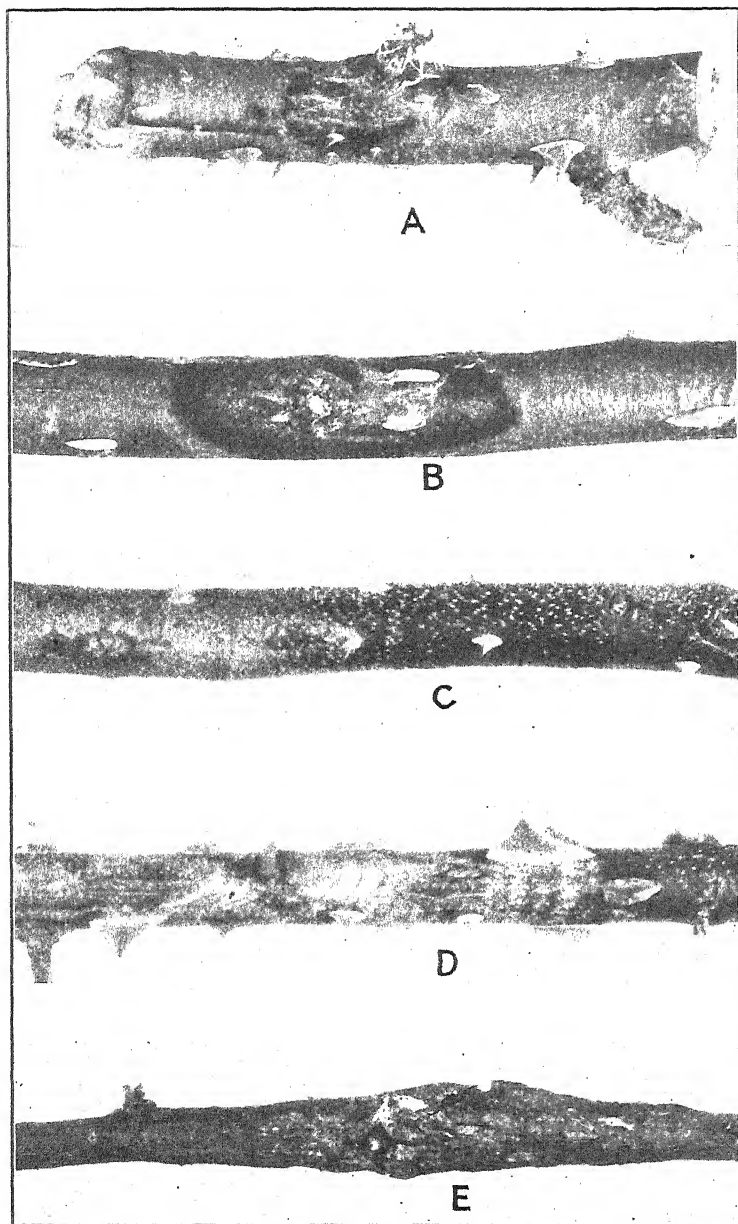
PLATE I

Portions of rose canes affected with *Botryosphaeria ribis* G. & D.

A, B.—Cankers as observed on March 21, 1923. The causal parasite has apparently entered through the small shoots at the center of the lesions.

C, D.—Blighted canes on June 28, 1923. The pycnidial pustules of the fungus appear as lighter colored areas distributed over the surface of the bark.

E.—Cankered portion of a cane of *Rosa setipoda* showing enlarged stem with the bark very much roughened and broken. Specimen received from the Federal Horticultural Board, October, 1922.



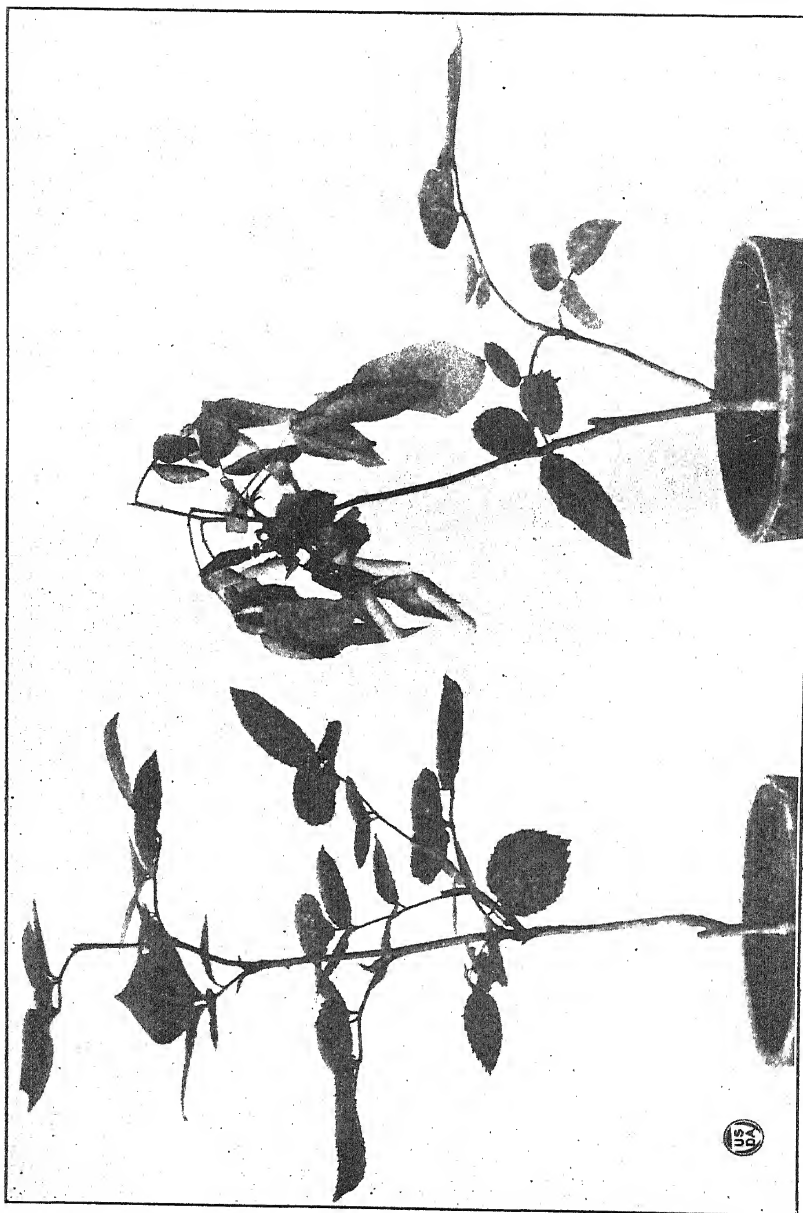




PLATE 2

On the right, rose plant of the variety Columbia four weeks after inoculation with *Botryosphaeria ribis* G. & D. The midrib of a leaf from the healthy branch crosses the main stem near the point of inoculation. Control on the left.



# ELEMENTAL COMPOSITION OF THE CORN PLANT<sup>1</sup>

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## INTRODUCTION

Our knowledge concerning the elemental composition of many of the more common agricultural plants is rather limited and fragmentary. The data on this subject have been obtained frequently from material that has been collected under no stated conditions and the earlier determinations are now open to criticism on account of the questionable accuracy of the methods of analysis employed. An accurate elemental analysis of a few mature plants of a given crop grown under well-defined conditions of soil and climate should furnish data from which the amount of the various elements removed from the soil by the crop could be fairly accurately estimated. For this reason it was thought advisable, in some of the experimental work with corn at the Kansas Agricultural Experiment Station, to make an elemental analysis of the plant. With this in view material was collected from mature Pride of Saline corn plants grown in the field at Manhattan, Kans., during the summer of 1920.

## CULTURAL METHODS

The plants used in this experiment were grown on plots that had been continuously cropped to corn for about 10 years. The soil was a fertile sandy loam and showed little difference in texture in the first 4 feet. The ground was plowed to a depth of 6 inches in the late fall and received no other cultivation until the following spring, when it was worked into good condition just previous to planting. The seed was surface planted on May 12 in rows 42 inches apart, and after the plants had reached a height of about 3 inches, they were thinned in the row to a distance of 2 feet between each plant. The plot was kept free from weeds by hoeing, and with the exception of a shallow cultivation on June 3 the soil was not stirred during the growing season. Since no visible signs of wilting were evident at any time during the summer, it is assumed that the supply of water in the soil was adequate. A summary of the general climatic conditions prevailing during the growing season is given in Table I.

<sup>1</sup> Received for publication Mar. 1, 1924. Published with the approval of the Director as paper No. 279, Department of Botany, and paper No. 106, Department of Chemistry, Kansas Agricultural Experiment Station.

TABLE I.—Summary of the climatic conditions at Manhattan, Kans., for the growing season of 1920

Month.	Days (inclusive).	Air temperature (° F.).					Precipitation.	Evaporation from free water surface.
		Average of—			Maxi- mum.	Mini- mum.		
		Mean.	Maxi- mum.	Mini- mum.				
							<i>Inches.</i>	<i>Inches.</i>
May	1-5	61	71	51	74	42	.....	0.568
Do.	6-10	61	73	50	83	46	0.64	.783
Do.	11-15	59	68	51	81	40	.25	.713
Do.	16-20	61	72	51	85	45	.04	.591
Do.	21-25	70	79	61	88	47	.48	.801
Do.	25-31	69	79	58	92	54	.17	.810
June	1-5	65	77	54	82	40	.34	1.077
Do.	6-10	78	90	67	98	58	.....	1.405
Do.	11-15	84	96	73	99	72	.....	1.983
Do.	16-20	68	78	59	91	55	.04	1.253
Do.	21-25	70	87	55	90	47	.76	1.418
Do.	26-30	84	79	61	98	69	.82	1.776
July	1-5	83	96	70	100	61	.02	1.339
Do.	6-10	71	85	57	89	54	.15	1.422
Do.	11-15	77	90	65	95	55	1.02	1.520
Do.	16-20	79	93	65	97	61	.....	1.290
Do.	21-25	82	97	67	105	63	.....	1.725
Do.	26-31	72	85	59	94	51	3.64	1.143
August	1-5	74	86	63	90	58	.....	1.128
Do.	6-10	79	94	61	96	57	.....	1.080
Do.	11-15	70	84	56	90	47	.12	1.232
Do.	16-20	71	87	53	91	48	5.18	.857
Do.	21-25	68	79	58	85	51	.....	1.172
Do.	26-31	73	84	62	92	56	.87	1.306

## COLLECTION AND PREPARATION OF MATERIAL

## STEMS, LEAVES, AND GRAIN

Five plants were selected and harvested on September 2, when the grains were in the late-dough stage and were well glazed and dented (Pl. 1, A). At this stage of development it was thought that the plants had removed from the soil most of the minerals of which they were capable, although there would probably be some slight rearrangement of materials in the various plant parts before full maturity. Furthermore, at this stage the leaves were all attached to the plants (Pl. 1, B) and green, so that little or no material had been lost from them by leaching. It is worthy of note that a gentle rain fell during the night of September 1, so that the leaves and stems were especially free from any adhering dirt when they were harvested. The plants were cut off at the surface of the ground and their green weight at once determined. A general description of each of the five plants at the time of harvesting is given in Table II.

As soon as the green weight of each plant was determined, the stem, leaves, and grain were separated, ground or cut into convenient pieces, and placed in a hot air drying oven at 105° C. for 24 hours. The material was then transferred to glass jars and sealed until it was ready to be

pulverized for chemical analysis. In the preparation of material the husks were placed with the leaves and the tassel with the stem. The cobs of the five plants were ground together and the analysis made of this mixture, so that the variation in the composition of these organs was not determined.

TABLE II.—General description of the five corn plants at the time of harvesting

Plant No.	Total green weight.	Dry weight of—					Percentage of moisture in plant.	Height of plant.	Length of ear.	Number of leaves.
		Entire plant, excluding the roots.	Stems and tassel.	Leaves <sup>a</sup> and husks.	Grain.	Cob.				
	Grams.	Grams.	Grams.	Grams.	Grams.	Grams.		Inches.	Inches.	
1.....	2,540	776.3	154.3	209.4	320.0	92.6	69.4	102	12	15
2.....	2,923	791.1	191.2	252.4	272.2	75.3	72.9	96	13	16
3.....	2,277	659.5	161.0	195.8	232.0	70.7	71.0	101	12	14
4.....	2,716	745.8	233.0	235.5	212.0	65.3	72.5	108	11	14
5.....	3,095	899.3	263.5	279.7	268.8	87.3	70.9	120	13	17
Average.	2,710	774.4	200.6	234.6	261.0	78.2	71.4	105	12	15

<sup>a</sup> Including sheaths.

#### ROOTS

It is practically impossible to collect all of the roots of a corn plant grown under crop conditions in the field since one is unable to distinguish or separate the finer roots that are interwoven with those of the adjacent plants. In order to obtain the entire root system, plants of the same variety of corn as that grown in the field were grown singly in soil in large galvanized iron cans. These cans contained a sufficient volume of soil to grow plants to maturity that in appearance were the equal in every regard of those growing under the conditions of the surrounding field. The methods used in growing plants in such containers have been previously described by Miller (8, 9)<sup>2</sup> and will not be discussed here. When the plants in the cans had reached maturity, the aerial parts were harvested and discarded and the roots collected in the following manner: The soil contained in the can was emptied upon a cleared space and the larger roots removed by careful sorting. The soil with the smaller roots was then placed in vessels and covered with a large excess of water which was stirred vigorously until the soil had disintegrated into fine particles. As soon as the stirring ceased, the soil settled to the bottom of the vessels while the remnants of roots floated to the surface and were removed by skimming with a fine sieve. The process was repeated several times until all the roots had been separated from the soil. The roots thus obtained were washed carefully until, in so far as could be seen, they were free from all soil particles, dried in a hot-air oven at 105° C. for 24 hours, and stored in sealed jars until chemical analyses could be made. A representative root system of one of the five obtained after this manner is shown in Plate 1, C.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 859.

## CHEMICAL METHODS

The chemical determinations of the various elements with the exception of sulphur and phosphorus were made, in general, according to the methods for the analysis of plant material recommended by the Association of Official Agricultural Chemists. The sulphur and phosphorus were determined after the following method described by Latshaw (7). One to two grams of the sample, or a sufficient amount to give a precipitate of not less than 30 mgm. of barium sulphate, were weighed into a 250 cc. low form Pyrex beaker. To this were added 7.5 cc. of a magnesium nitrate solution,<sup>3</sup> care being taken that all the material was brought in contact with the solution and heated on an electric hot plate (180° C.) until no further action took place. The beaker was transferred while hot to an electric muffle and allowed to remain at low heat (muffle not showing any red) until the charge was thoroughly oxidized and no black particles remained. If necessary, the charge was broken up and again returned to the muffle. The beaker was removed from the muffle and allowed to cool. Water was added, then hydrochloric acid in excess, the solution brought to a boil, filtered and washed thoroughly. This solution was then diluted to 200 cc., heated to boiling, and a 10 per cent barium-chlorid solution added in small quantities until no further precipitate was formed. The boiling was continued for five minutes, after which the liquid was allowed to stand for five hours or longer in a warm place. The liquid was then decanted on an ashless filter or a tared Gooch crucible previously heated, the precipitate treated with 15 to 20 cc. of boiling water, transferred to the filter and washed free of chlorids with boiling water. The precipitate was ignited and weighed as barium sulphate. The filtrate obtained in the sulphur determination was evaporated to 75 cc. and the phosphorus determined by the method recommended by the Association of Official Agricultural Chemists.

The figures in the various tables representing the amounts of oxygen include the oxygen obtained by the usual procedure in organic analysis plus the oxygen that was a part of the various mineral elements of the ash when they were converted to their oxids. The figures thus represent the oxygen of both organic and inorganic combination.

The determinations were all made in duplicate or triplicate and whenever any striking differences appeared in the analysis of the individual plants a careful redetermination was made to verify the results. Any marked differences recorded in the composition of the same organ of the different plants are due to individual variations and not to discrepancies in the chemical methods used.

## DISCUSSION OF EXPERIMENTAL DATA

In order to determine the variation in the composition of the different plants and their organs a separate analysis was made of the leaves, stem, and grain of each of the five plants. A separate analysis was also made of each of the five root systems. Determinations were made for carbon, oxygen, hydrogen, nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, iron, silicon, aluminum, chlorin, and manganese. The amount of each of the elements that compose the leaves, stem, and grain of each of the five plants is expressed in percentage of dry weight and in the actual amount, in grams, in Table III.

<sup>3</sup> Made by dissolving 300 gm. of calcined magnesia in nitric acid, avoiding an excess of the latter. Calcined magnesia is then added slightly in excess, the solution boiled, filtered from the excess of magnesia, iron alumina, etc., and diluted to two liters.

In Table IV the composition of the five root systems is expressed in the same manner. The data in Table III shows a striking uniformity in the elemental composition, expressed in percentage of dry weight, of the leaves, stem and grain of each of the five plants analyzed. The only marked exceptions in this regard were the rather wide variations in the percentages of oxygen and chlorin. This was true, however, only of the stems and leaves since the percentages of these two elements in the grain were very uniform. The percentages of several of the mineral elements in the five root systems showed considerable variation which may have been due, in part, to the unequal distribution of minute particles of soil adhering to or embedded in the exterior tissue of the roots.

Although the percentage elemental composition is uniform in a like organ of the different plants, the actual amount of a given element expressed in grams varies considerably, as shown in the latter part of Table III. This is due to the fact that plants which are grown under the same conditions in the field and which seem to be uniform in size and general appearance show marked variations in dry weight and in the distribution of this matter in the various organs of the plants.

#### DISTRIBUTION OF THE ELEMENTS IN THE ORGANS OF THE PLANT

The total dry weight of the leaves, stem, grain and cobs of each of the five corn plants analyzed was 776.3, 791.1, 659.5, 745.8 and 899.3 gm., respectively. The average amount of dry matter in the aerial parts of the plants was thus 774.4 gm., of which 30.2 per cent was in the leaves, 26 per cent in the stem, 33.7 per cent in the grain and 10.1 per cent in the cob. This proportion of dry matter in the various organs corresponds closely to the observations of Smith (11) on corn grown in Michigan. He found that 22 per cent of the dry matter of the plant above ground was in the leaves, 32 per cent in the stalks and 46 per cent in the ears. Table IV shows that the dry weight of each of the five root systems isolated was, respectively, 59.5, 62.4, 60.7, 53.9, and 66.0 gm., with an average weight of 60.5 gm. The weight of the dry matter of the roots was thus 7.81 per cent of the dry weight of the leaves, stem and ear. The weight of the roots obtained in this experiment is in accord with unpublished data that have been obtained in numerous experiments with the corn plant at the Kansas Agricultural Experiment Station. These data show that the dry weight of the roots of a mature Freed White Dent, Kansas Sunflower, Reid Yellow Dent or Pride of Saline corn plant was between 7 and 8 per cent of the dry weight of the portion of the plant above ground.

The values for the weight of the dry matter of the roots of corn are much higher than those given by Hornberger (3) and Schweitzer (10). Their results show that the dry weight of the roots of mature corn plants grown in the field did not exceed 3 per cent of the dry weight of the plants. They state, however, that the methods used by them to isolate the root systems were unsatisfactory since there was no degree of certainty that they had obtained all the roots.

The average percentage composition of the different organs of the five plants is shown in the first half of Table V. The average weight in grams of the elements that compose, respectively, the stem, leaves, grain, cob, and roots is shown in the last half of Table V. These values expressed in grams were obtained by multiplying the average weight of the organs by their average percentage composition. The total weight of each of the elements that make up the mature plant is expressed graphically in Figure 1.

TABLE III.—Elemental composition of the leaves, stems, cobs, and grain of five *Pride of Saline* corn plants grown in the field at Manhattan, Kans., in 1920

Plant No.	Organ.	Dry weight.	ELEMENTS.													
			Carbon.	Oxygen.	Hydro-gen.	Nitro-gen.	Phos-phorus.	Potas-sium.	Cal-cium.	Magne-sium.	Sul-phur.	Iron.	Silicon.	Alumi-num.	Chlo-rin.	Manga-nese.
			PERCENTAGE OF THE ELEMENTS, DRY BASIS.													
1	Leaves.....	41.18	45.28	5.75	1.16	0.169	1.57	0.56	0.25	0.25	0.070	2.44	0.075	0.34	0.031	
2	do.....	40.85	44.49	6.20	1.40	.202	1.57	.38	.23	.23	.073	2.20	.086	.43	.023	
3	do.....	42.25	43.35	5.77	1.43	.225	1.32	.46	.20	.23	.081	2.69	.074	.076	.036	
4	do.....	40.85	44.24	5.82	1.21	.186	1.54	.49	.17	.24	.058	2.57	.071	.154	.044	
5	do.....	41.22	41.95	5.78	1.32	.256	1.38	.47	.20	.23	.070	3.05	.063	.115	.025	
1	Stem.....	43.96	43.91	5.66	.88	.073	1.64	.14	.14	.15	.052	.43	.016	.40	.016	
2	do.....	44.08	42.90	5.83	.94	.093	1.09	.19	.18	.13	.046	.30	.014	.40	.010	
3	do.....	46.35	42.50	6.00	.50	.072	1.12	.14	.12	.16	.046	.41	.012	.13	.023	
4	do.....	44.35	45.00	5.93	.85	.081	1.13	.20	.14	.17	.046	.49	.012	.072	.017	
5	do.....	43.80	45.20	6.08	1.02	.124	1.10	.20	.21	.17	.074	.47	.012	.12	.018	
1	Grain.....	45.25	45.05	7.01	2.14	.31	.37	.035	.20	.12	.049	.018	.020	.026	.027	
2	do.....	44.48	45.42	7.02	2.14	.33	.31	.028	.20	.18	.030	.014	.036	.031	.044	
3	do.....	44.75	45.31	6.93	2.22	.33	.43	.023	.19	.12	.028	.021	.033	.031	.050	
4	do.....	45.10	44.67	6.94	2.24	.40	.45	.021	.19	.14	.055	.016	.014	.031	.043	
5	do.....	44.00	46.04	6.92	2.03	.34	.34	.019	.21	.12	.055	.011	.013	.046	.023	
	Cobs.....	45.75	45.89	6.36	1.38	.094	.46	.022	.11	.021	.025	.133	.052	.12	.031	
TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.																
1	Leaves.....	209.4	86.23	94.81	12.04	2.42	0.353	3.28	1.17	0.523	0.523	0.146	5.10	0.157	0.711	0.065
2	do.....	252.5	103.10	112.29	15.64	3.53	.510	3.96	.96	.581	.581	.184	5.55	.217	1.085	.058
3	do.....	195.8	82.72	84.87	11.29	2.79	.440	2.58	.90	.391	.450	.158	5.27	.144	.142	.070
4	do.....	235.5	96.20	104.18	13.71	2.84	.438	3.62	1.15	.400	.505	.136	6.05	.167	.362	.104
5	do.....	279.7	115.29	117.33	16.16	3.69	.716	3.86	1.31	.559	.643	.195	8.53	.176	.321	.069



1	Stems.	154.3	67.83	67.75	8.73	1.36	.112	2.53	.21	.210	.231	.086	.66	.024	.617	.024
2	do.	101.2	84.28	82.02	11.14	1.79	.177	2.08	.36	.344	.248	.076	.57	.026	.760	.019
3	do.	161.0	74.62	68.42	9.66	.81	.115	1.80	.23	.193	.257	.074	.66	.019	.209	.037
4	do.	233.0	103.33	104.85	13.81	1.98	.188	2.63	.46	.326	.396	.107	1.14	.027	.167	.039
5	do.	263.5	115.40	119.10	16.02	2.68	.326	3.05	.52	.553	.447	.194	1.23	.031	.316	.047
1	Grain.	320.0	144.80	144.16	23.43	6.84	.990	1.18	.112	.640	.384	.156	.057	.064	.083	.086
2	do.	272.2	121.07	123.63	10.10	5.82	.890	1.38	.076	.544	.489	.082	.038	.098	.084	.119
3	do.	232.0	103.82	105.11	16.07	5.15	.760	1.00	.053	.440	.278	.065	.049	.076	.072	.116
4	do.	212.0	95.61	94.70	14.71	4.74	.850	.96	.045	.406	.299	.117	.034	.029	.066	.092
5	do.	268.8	118.27	123.75	18.60	5.45	.910	.91	.051	.564	.322	.147	.029	.035	.123	.061
1	Cobs.	92.6	42.36	42.49	5.88	1.27	.087	.425	.020	.101	.019	.023	.123	.048	.111	.029
2	do.	75.3	34.44	34.55	4.78	1.03	.071	.346	.017	.083	.016	.018	.100	.039	.090	.023
3	do.	70.7	32.34	32.44	4.49	.98	.066	.325	.015	.078	.015	.018	.004	.037	.085	.022
4	do.	65.3	29.87	29.96	4.15	.90	.061	.30	.014	.072	.014	.010	.086	.034	.078	.020
5	do.	87.3	39.93	40.06	5.55	1.20	.082	.40	.019	.096	.018	.022	.116	.045	.104	.027

TABLE IV. Elemental composition of the roots of five *Pride of Saline* corn plants grown in containers at Manhattan, Kans., in 1920

Plant.	Dry weight.	ELEMENTS.													
		Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorine.	Manganese.
PERCENTAGE OF THE ELEMENTS, DRY BASIS.															
A.....		42.59	46.14	5.93	1.27	0.141	0.27	0.58	0.18	0.28	0.43	6.63	0.89	0.12	0.045
B.....		43.37	45.80	5.79	1.24	.122	.57	.56	.17	.25	.44	4.89	.94	.09	.088
C.....		43.35	43.15	6.01	1.27	.121	.56	.64	.18	.23	.45	3.38	.89	.10	.056
D.....		42.65	42.05	5.56	1.40	.101	.37	.72	.15	.24	.50	4.17	1.15	.12	.089
E.....		39.70	40.78	5.33	1.16	.113	.65	.56	.16	.23	.76	3.13	1.02	.11	.055
TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.															
A.....	59.5	25.28	27.45	3.53	0.75	0.083	0.160	0.345	0.107	0.166	0.255	3.94	0.529	0.071	0.027
B.....	62.4	27.06	28.57	3.61	.77	.076	.355	.349	.106	.156	.274	3.05	.586	.056	.055
C.....	60.7	26.31	26.19	3.64	.77	.073	.339	.388	.109	.139	.273	2.95	.540	.061	.034
D.....	53.9	22.98	22.66	3.00	.75	.054	.199	.388	.081	.129	.269	2.24	.619	.064	.048
E.....	66.0	26.20	26.91	3.51	.76	.074	.429	.369	.105	.151	.501	2.06	.673	.073	.036

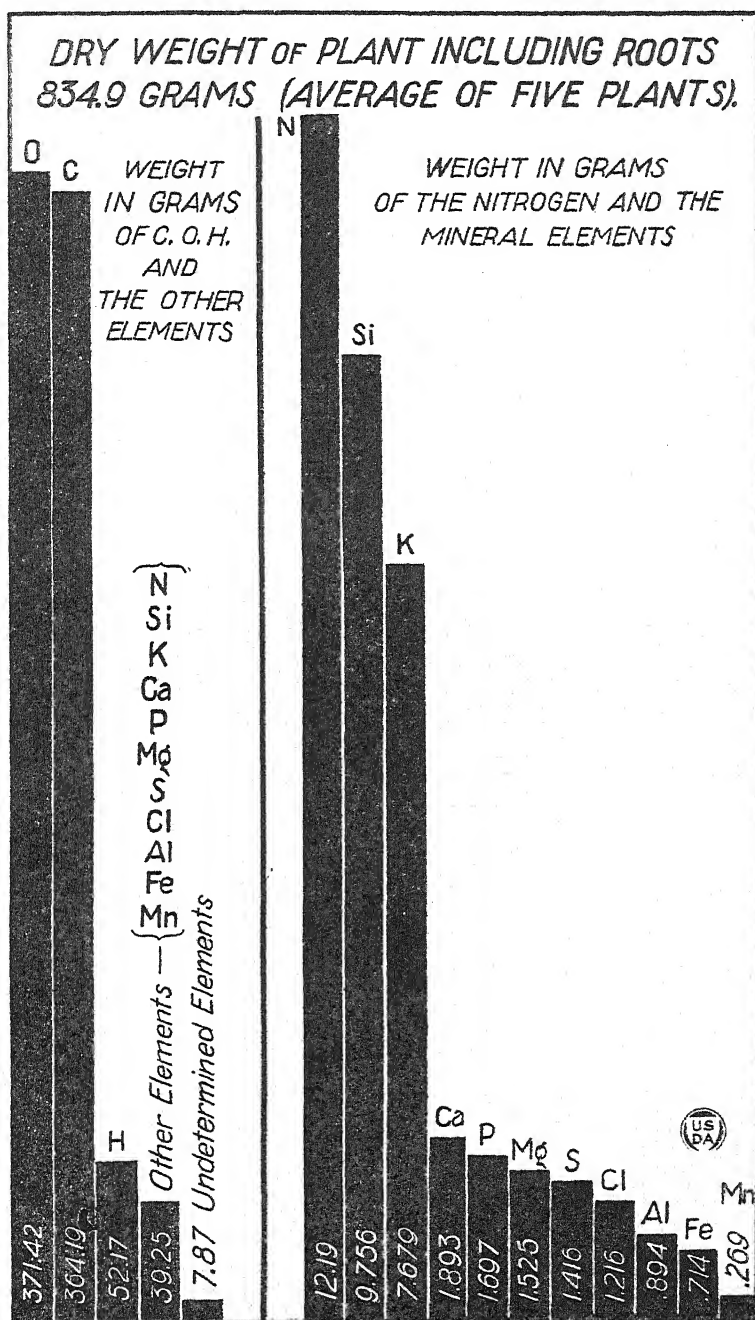


FIG. 1.—Relative amount of the elements that composed the dry matter of Pride of Saline corn grown at Manhattan, Kans., in 1920.

TABLE V.—Average amount of the elements in the leaves, stems, cobs, grain, and roots of five *Pride of Saline* corn plants grown at Manhattan, Kans., in 1920

Organ.	Dry weight.	ELEMENTS.													
		Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorine.	Manganese.
PERCENTAGE OF THE ELEMENTS, DRY BASIS.															
Leaves.....	.....	41.27	43.86	5.86	1.30	0.207	1.48	0.47	0.21	0.24	0.070	2.59	0.074	0.222	0.043
Stems.....	.....	44.51	43.90	5.90	.84	.089	1.23	.17	.16	.16	.052	.42	.013	.224	.017
Grain.....	.....	44.72	45.30	6.06	2.15	.34	.42	.025	.20	.14	.043	.016	.023	.033	.037
Roots.....	.....	42.31	43.58	5.72	1.27	.120	.48	.61	.17	.25	.52	4.44	.98	.11	.066
Cobs.....	.....	45.75	45.89	6.36	1.38	.094	.46	.022	.11	.021	.025	1.33	.052	.12	.031
TOTAL WEIGHT OF THE ELEMENTS IN GRAMS.															
Leaves.....	234.60	96.82	102.89	13.74	3.05	0.486	3.47	1.101	0.493	0.563	0.164	6.08	0.174	0.521	0.075
Stems.....	200.60	89.29	88.06	11.83	1.68	.178	2.47	.341	.321	.321	.104	.84	.026	.449	.034
Grain.....	261.00	116.72	118.23	18.17	5.61	.887	1.99	.065	.522	.365	.112	.042	.060	.086	.096
Roots.....	60.50	25.59	26.36	3.46	.77	.072	.290	.369	.103	.151	.315	2.69	.593	.066	.040
Cobs.....	78.20	35.77	35.88	4.97	1.08	.074	.359	.017	.086	.016	.019	.104	.041	.094	.024
Average total weight...	834.90	364.19	371.42	52.17	12.19	1.697	7.679	1.893	1.525	1.416	.714	9.756	.894	1.216	.269

Taking the average dry weight of the aerial parts of the plants as 774.4 gm. and the average dry weight of the roots as 60.5 gm., the average total dry weight of a Pride of Saline corn plant grown under the conditions of this experiment amounted to 834.9 gm. Of this total amount of dry matter 28.10 per cent was in the leaves, 24.02 per cent in the stem, 31.26 per cent in the grain, 9.37 per cent in the cob and 7.25 per cent in the roots. Of this total dry weight of the corn plant, carbon composed 43.62 per cent, oxygen 44.48 per cent, hydrogen 6.24 per cent, nitrogen 1.46 per cent, phosphorus 0.204 per cent, potassium 0.919 per cent, calcium 0.226 per cent, magnesium 0.182 per cent, sulphur 0.169 per cent, iron 0.085 per cent, silicon 1.17 per cent, aluminum 0.107 per cent, chlorine 0.145 per cent and manganese 0.032 per cent. Approximately 1 per cent of the total dry weight was thus to be accounted for by the undetermined elements of which, no doubt, sodium represented a considerable proportion. Carbon, oxygen and hydrogen made up 94.34 per cent of the dry weight of the plant. Since 1.46 per cent of the dry matter of the plant was due to nitrogen, the mineral elements in the plant represented only slightly more than 4 per cent of its entire dry weight.

The percentage of the various elements that composed the plants in these analyses corresponded closely to the figures given by other investigators. Hornberger (3) worked with Badischer Early corn and made chemical analyses of the plants at seven-day periods throughout the growing season. His analyses show that at the time of maturity calcium, potassium, phosphorus, magnesium, sulphur, silicon, iron and nitrogen made up, respectively, 0.51, 1.19, 0.27, 0.42, 0.08, 0.40, 0.05, and 1.54 per cent of the dry weight of the plants. The figures given by Vivian (12, p. 9-11) show that carbon, oxygen and hydrogen made up 92.7 per cent of the dry weight of the corn plant which was ready to be cut for the shock. His results for the percentages of the various mineral elements were practically the same as those reported by Hornberger (3). Jones and Huston (5) found that potassium, phosphorus and nitrogen made up, respectively, 0.828, 0.193, and 1.17 per cent of the dry weight of the stems, leaves and ears of Riley's Favorite variety of corn when the grain was hard and the plant ready to be cut.

The percentage distribution of the 14 elements in the different parts of the plant is shown in Table VI.

TABLE VI.—Percentage distribution of the different elements in the leaves, stem, grain, cob, and roots of Pride of Saline corn grown at Manhattan, Kans., in 1920

Organ.	Elements.													
	Carbon.	Oxygen.	Hydrogen.	Nitrogen.	Phosphorus.	Potassium.	Calcium.	Magnesium.	Sulphur.	Iron.	Silicon.	Aluminum.	Chlorine.	Manganese.
Leaves.....	26.58	27.70	26.33	25.01	28.63	45.18	58.16	32.32	39.75	22.96	62.32	19.46	42.84	27.88
Stems.....	24.51	23.70	22.67	13.78	10.48	32.16	18.01	21.04	22.66	14.57	8.61	2.90	36.92	12.64
Grain.....	32.04	31.83	34.82	46.01	52.26	14.19	3.43	34.22	25.77	15.68	.43	6.71	7.07	35.68
Roots.....	7.02	7.09	9.52	6.31	4.24	3.76	19.49	6.75	10.66	44.11	27.57	66.33	5.42	14.87
Cobs.....	9.82	9.66	6.63	8.85	4.36	4.67	.89	5.63	1.12	2.66	1.06	4.58	7.75	8.92

Some of the more important facts shown in this table should be mentioned in the text. The grain and cob contained approximately 55 per cent of the total nitrogen, while 25 per cent of this element occurred in the leaves. The phosphorus was distributed in the same relative

proportion, since 56 per cent of the total phosphorus was in the grain and cob and 29 per cent in the leaves. Approximately 45 per cent of the potassium occurred in the leaves, 32 per cent in the stem and only 19 per cent in the grain and cobs. More than 58 per cent of the calcium was in the leaves, while the stems and roots contained 18 and 19 per cent, respectively. The proportion of calcium in the ear was small and amounted to slightly more than 4 per cent of the total calcium in the plant. In contrast to the calcium, more than 39 per cent of the magnesium occurred in the grain and cob while the proportion of this element in the leaves and stem amounted to 32 and 21 per cent, respectively. The leaves contained 39 per cent of the sulphur, the stem 22 per cent and the grain and cob 26 per cent. Approximately 22 per cent of the iron was found in the leaves, while the stem and grain each contained 15 per cent of this element. The leaves contained the greater part of the silicon, since over 62 per cent of the total amount of this element occurred in them. The ear contained about 1.5 per cent of the silicon and of this amount less than one-half per cent occurred in the grain. The leaves and the stem contained most of the chlorine; 42 per cent of this element occurred in the former and 36 per cent in the latter. Approximately 44 per cent of the manganese occurred in the grain and cob and over 27 per cent in the leaves. The roots contained 6.3 per cent of the nitrogen, 4.2 per cent of the phosphorus, 3.7 per cent of the potassium, 19.5 per cent of the calcium, 6.7 per cent of the magnesium, 10.6 per cent of the sulphur, 44.1 per cent of the iron, 27.5 per cent of the silicon, 66.4 per cent of the aluminum, 5.4 per cent of the chlorine and 14.8 per cent of the manganese. The relatively large proportions of calcium, iron, silicon and aluminum in the roots was due in part, probably, to the minute soil particles that were partially embedded in the surface of the roots and were not removed in the preparation of the material for analyses.

#### WEIGHT OF THE ELEMENTS REMOVED FROM THE AIR AND SOIL BY AN ACRE CROP OF CORN

Since so many factors can influence the yield and composition of plants, any data concerning the amount of the various elements removed per acre for a given crop are applicable only for the cultural and climatic conditions under which the plants were grown. The recent work of Duley and Miller (1) concerning the effect of the supply of nutrients upon the character and composition of the corn plant especially emphasizes this fact. In the experiment herein reported, the plants were grown in 42-inch rows and the plants in the row thinned to a distance of 2 feet. If the stand were perfect under these conditions, there would be 6,270 plants per acre, but in order to allow some leeway 6,200 plants were considered as the stand per acre in estimating the yield of dry matter. The total dry matter produced by 6,200 plants, the average dry weight of which, including the roots, was 834.9 gm., would amount to 11,389 pounds per acre. Since the dry matter of the roots weighed 60.5 gm. per plant, the dry matter produced per acre by them would amount to 825 pounds. The total dry weight of the plants above ground would thus amount to 10,564 pounds per acre. The average dry weight of the lower foot of the stem was determined in order to ascertain the amount of dry matter left on the field by the stubs when the plants were cut at a height of 1 foot from the ground. The average weight of the lower foot of the stem was 28.3 gm. If 6,200 plants were considered as

the stand per acre, the weight of dry matter remaining in the stubs would be 386 pounds. The total weight of dry matter left in the field by the stubs and roots amounted, according to these estimations, to 1,211 pounds per acre, or approximately 10.6 per cent of the total dry matter produced. The total dry matter produced per acre, exclusive of the roots and the stubs 1 foot in height, amounted to 10,178 pounds per acre. This yield of dry matter of the stems, leaves, and ears of the corn plant is considerably higher than that reported by other investigators, but it is difficult to make comparisons since in some cases the variety of corn grown and the height of the stalks when cut at harvesting were not stated. Ladd (6) reported a yield of 7,918 pounds of dry matter per acre for the King Phillip variety at the Geneva (N. Y.) Agricultural Experiment Station, but did not mention how much of the stalk was left on the stub. Smith (11) obtained a yield of 8,020 pounds of dry matter per acre at the Michigan Experiment Station, but no record of the variety used or the method of harvesting is given. Schweitzer (10) at the Missouri Experiment Station considered 6,528 plants to the acre and estimated the total yield of dry matter at 7,892 pounds, but neglected to state the variety of corn used. Jones and Huston (5) estimated the yield of dry matter of Riley's Favorite grown at the Indiana Experiment Station at 9,412 pounds when the plants were harvested at the surface of the ground and when 10,000 plants were considered as the stand per acre. Ince (4) states that the average yield of dry matter of the ears, stems, and leaves of numerous varieties of corn grown at the North Dakota Experiment Station was 6,130 pounds, but he does not mention how much of the stalk was left on the ground in harvesting.

The estimated weight in pounds of the elements removed per acre from the soil and the air by the entire corn plant and by each of the several organs or parts is shown in Table VII.

TABLE VII.—*Estimated weight in pounds of the elements removed per acre from the air and soil by 6,200 Pride of Saline corn plants grown at Manhattan, Kans., in 1920*

Element.	Weight in pounds of the elements in—									
	Entire plant.	Roots.	Aerial parts.	Stubs 1 foot high.	Roots and stubs 1 foot high.	Grain.	Cobs.	Cobs and grain.	Leaves.	Stems.
Carbon.....	4,967.55	349.04	4,618.51	171.59	520.63	1,592.06	487.90	2,079.96	1,320.62	1,217.91
Oxygen.....	5,066.17	359.55	4,706.62	169.27	528.82	1,612.65	489.40	2,102.05	1,403.41	1,201.13
Hydrogen.....	711.60	47.19	664.41	22.64	69.83	247.83	67.79	315.62	187.41	161.36
Nitrogen.....	166.27	10.50	155.77	3.22	13.72	76.52	14.73	91.25	41.60	22.91
Phosphorus.....	23.15	.98	22.17	.04	1.02	12.09	1.01	13.10	6.63	2.42
Potassium.....	104.74	3.95	100.79	4.74	8.69	14.86	4.89	19.75	47.33	33.69
Calcium.....	25.82	5.03	20.79	.65	5.68	.89	.23	1.12	15.02	4.65
Magnesium.....	20.80	1.40	19.40	.61	2.01	7.12	1.17	8.29	6.72	4.37
Sulphur.....	19.31	2.06	17.25	.61	2.67	4.98	.22	5.20	7.68	4.37
Iron.....	9.74	4.30	5.44	.19	4.49	1.52	.20	1.78	2.23	1.41
Silicon.....	133.07	36.70	96.37	1.61	38.31	.42	1.42	1.84	82.93	11.45
Aluminum.....	12.19	8.09	4.00	.05	8.14	.81	.25	1.37	2.37	.35
Chlorine.....	16.59	.90	15.69	.84	1.74	1.17	1.58	2.45	7.11	6.12
Manganese.....	3.67	.55	3.12	.07	.62	1.30	.33	1.62	1.02	.40

These results were obtained by multiplying the weight of each of the different elements in the different organs, as shown in Table V, by 6,200 and reducing the results thus obtained to pounds. The results are self-explanatory and need not be discussed in detail in the text. The figures

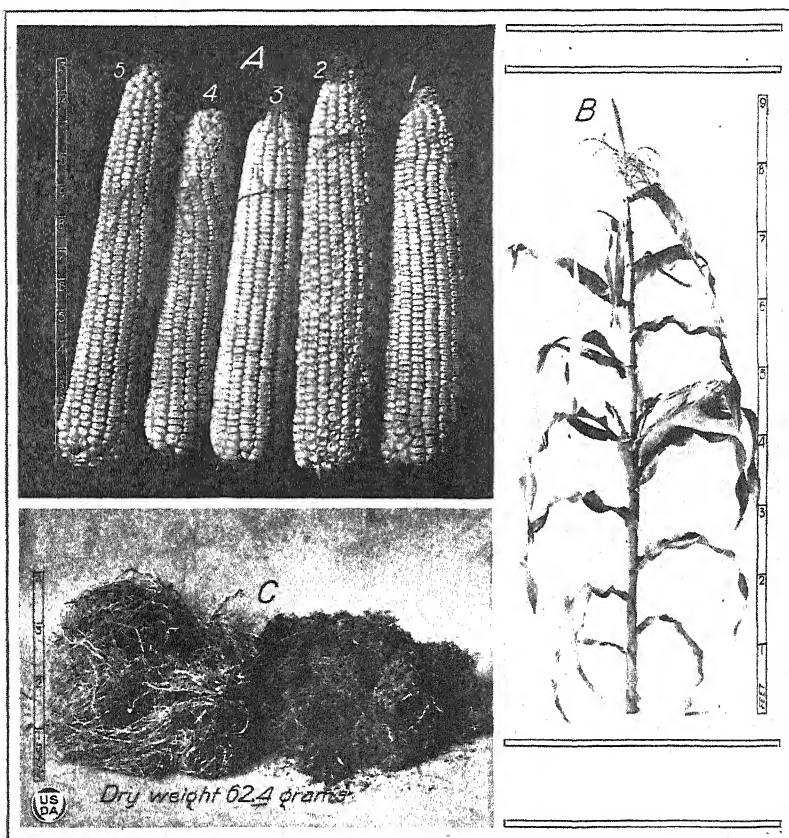
PLATE 1

A.—Ears of the five Pride of Saline corn plants that were used for the elemental analyses.

B.—Pride of Saline corn plant No. 4 showing the general appearance of the five plants used for the elemental analyses.

C.—General appearance of one (B, Table IV) of the five root systems of Pride of Saline corn plants used in the chemical analyses.







# WHEAT SCAB AND CORN ROOTROT CAUSED BY *GIBBERELLA SAUBINETII* IN RELATION TO CROP SUCCESSIONS<sup>1</sup>

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## INTRODUCTION

It is a well-known fact that corn, like other field crops, can not be grown year after year on the same soil without harmful effects. Winter wheat is a crop well adapted to the corn belt. Like corn, it is one of the most desirable crops from the standpoint of immediate financial returns. It, therefore, has become a common practice in the Corn Belt to include both corn and wheat in the rotation. In many cases wheat is sown directly after corn.

In preparing corn land for wheat several cultural methods are in common use, but very seldom is the field plowed before sowing the wheat. Winter wheat is often sown with a one-horse drill between the standing rows of corn in the early fall. After the ground has become frozen the corn is husked and the cornstalks are broken down by dragging a pole over them. In some localities the corn is husked early, the cornstalks broken down and cut up with a disk harrow, and the field sown with winter wheat in the fall or spring wheat in the early spring. In other sections the corn is cut and shocked, after which wheat is sown in the disked corn stubble. Another practice in rather general use is to cut the corn for silage and thus remove all of it, except the stubble, from the field before sowing the wheat. The wheat is commonly followed by a crop of oats, after which corn again is planted, or the wheat may be followed directly by corn. Although wheat is generally sown directly after corn, yet the crop sequence often is oats after corn and wheat after the oats. As will be pointed out later, this is a much more desirable crop sequence from the standpoint of the control of wheat scab (*Fusarium blight*).

In some regions a legume, such as clover or soybeans, frequently is included in the rotation. In sections where the farming has become more intensive or where the soils are less fertile, clover is sown every three or four years. This not only builds up the nitrogen content of the soil but tends to reduce the accumulation of soil-borne parasites.

<sup>1</sup> Received for publication Jan. 29, 1924. The investigations upon which this paper is based were conducted as a cooperative project between the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, the Wisconsin Agricultural Experiment Station, and Funk Bros. Seed Company, Bloomington, Ill.

## WHEAT SCAB IN RELATION TO PREVIOUS CROPPING

## REVIEW OF LITERATURE

Latta, Arthur, and Huston (15),<sup>2</sup> in 1891, mentioned the prevalence of scab on a plat where wheat had been grown continuously and on an adjacent plat where wheat and corn had been grown alternately for 11 years. This seems to be the first recorded observation on the relation of previous cropping to the occurrence of wheat scab. In 1909, Selby and Manns (19) also recorded observations on the relation of previous cropping to the production of wheat scab. They noticed that on the experimental plats that had been sown to wheat continuously for a number of years scab was worse than on the plats where the crops had been rotated. Some years later Bolley (3) emphasized the importance of rotating wheat with some other unrelated crop to keep down wheat scab. He recommended rotating with clover, alfalfa, grasses, potatoes, flax, and corn.

In 1918, Hoffer, Johnson, and Atanasoff (8) reported that there was a greater abundance of wheat scab in fields where wheat was grown immediately following corn that was infected with the *Fusarium* root and stalk rots. They found that wheat-scab infection may take place from *Gibberella* spores growing on old diseased cornstalks and that corn rootrot can be produced under laboratory conditions by *Gibberella* spores isolated from scabbed wheat. During the same year Hoffer and Holbert (7) reported a greater amount of scab when wheat was sown in infested corn fields.

In 1919, Holbert, Trost, and Hoffer (11) reported on the occurrence of wheat scab as affected by crop rotations. Their observations, which covered 28 fields, comprising 1,500 acres, confirmed previous observations that wheat following corn is scabbed more severely than that following any other crop.

Since then, reports have appeared by Johnson and Haskell (14, p. 24-26), Johnson and Dickson (13), Fromme (6, p. 141), and Adams (1) stating that wheat-scab infection is heaviest when wheat follows a corn crop.

SURVEY OF 1919<sup>3</sup>

In 1919 counts were made of wheat-scab infection following various common crops in seven different States. There was an unusual epidemic of scab during this season. Most of the wheat examined was in the hard-dough stage and some of it was ripe. Each percentage determination was made by counting a hundred heads along a drill row and breaking off all heads that showed the presence of scab. The number of scabbed heads picked off then gave the percentage of infected heads. At the close of each day, the heads thus collected were sent to the laboratory at Madison, Wis., for identification of the organisms. In making the scab counts, four or more, and often over a hundred, percentage determinations were made in different parts of each field.

In Wisconsin, where the grain had recently been cut, the percentage determinations were made from entire bundles. From four to nine bundles were selected from different parts of each field, each bundle containing over 2,000 heads.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," pp. 878-879.

<sup>3</sup> L. E. Compton, Junior Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, assisted in obtaining these survey data.

This survey was made primarily for accurate information on the distribution of wheat-scab, but notes were made also on a number of phases of the problem including the crop successions during the three previous years. As the same crop rotation usually is not followed on the different farms in any community, adjacent fields or fields close to each other were observed where wheat followed corn in some cases and in other cases followed other crops.

Wherever wheat followed corn in the various States, perithecia of *Gibberella saubinetii* (Mont.) Sacc. were found in abundance on the old cornstalks and stubble left in the field. To make certain of their identity, specimens were sent to the laboratory at Madison, Wis., from each farm where observations were made.

Table I gives a summary of the data collected in 1919. The difference between the percentage of heads scabbed when wheat followed corn as compared with the percentage when wheat followed some other crop is large enough in each State to be significant. A large number of wheat varieties are included in this summary. There is some varietal difference in the degree of susceptibility to scab, but the differences in respect to previous cropping are similar in all varieties and therefore all are summarized in one table.

Scab also affects other cereals, including rye and oats, but generally the infection is of less extent than in wheat. Table I shows not only that wheat-scab infection is most severe when wheat follows corn, but that it also is greater when wheat follows wheat, rye, or oats than when it follows clover or timothy. The last two crops usually are not attacked by *Gibberella saubinetii*, the chief causal organism of scab of cereals, although Selby and Manns (19) report a stem rot of clover produced probably by this parasite. Therefore, the disease is not so likely to be carried over from the previous year on the refuse from these two crops as on refuse from oats, wheat, and corn. These data show that, over a large area, wheat scab occurs most abundantly where wheat follows corn.

TABLE I.—Average percentages of wheat scab (head blight) in 1919 following different crops in seven States

State.	Number of counties surveyed.	Number of fields surveyed.	Average percentages of wheat scab after previous crop of—					
			Corn.	Wheat.	Rye.	Oats.	Clover.	Timothy.
Illinois.....	2	23	59	33	.....	33	22	.....
Indiana.....	10	47	39	25	30	16	20	.....
Iowa.....	9	27	71	49	.....	30	.....	.....
Minnesota.....	4	20	66	58	50	45	.....	5
Ohio.....	5	39	33	6	.....	11	1	17
Tennessee.....	5	16	21	4	1	.....	9	.....
Wisconsin.....	4	10	14	.....	.....	1	5	3
Average.....	.....	.....	43.3	29.2	27.0	22.7	11.4	8.3

#### SURVEY OF 1920

The survey of 1920, as summarized in Table II, was confined to McLean County, Ill. All of the fields surveyed were on the same type of soil, in a more or less contiguous area, and all were sown with Turkey

wheat. While only a small wheat acreage was surveyed as compared with that of the previous year, the conditions under which the crop was grown were very much more uniform, so that in many ways the data are more significant than those obtained in 1919.

TABLE II.—Percentages of scab (head blight) in Turkey wheat following different crops, in McLean County, Ill., in 1920

Field Number.	Size of field.	Previous crop.	Percentages of scab at different locations in fields.													Grand average following each crop.
			1	2	3	4	5	6	7	8	9	10	11	12	Average.	
	Acres.		Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
107	80	Corn.....	6.0	10.0	4.0	5.0	2.0	3.0	6.0	5.0	.....	.....	.....	.....	5.1	4.5
479	30	do.....	8.0	3.0	3.0	10.0	2.0	2.0	5.0	.....	.....	.....	.....	.....	4.7	
473	10	do.....	2.0	4.0	2.0	4.0	.....	.....	.....	.....	.....	.....	.....	.....	3.0	
475	10	do.....	3.0	3.0	5.0	3.0	5.0	3.0	.....	.....	.....	.....	.....	.....	3.7	
472	10	do.....	3.0	4.0	4.0	5.0	3.0	3.0	.....	.....	.....	.....	.....	.....	3.7	
660	40	do.....	5.0	11.0	9.0	9.0	4.0	10.0	8.0	7.0	.....	.....	.....	.....	7.9	
167	60	do.....	5.0	9.0	4.0	3.0	7.0	2.0	.....	.....	.....	.....	.....	.....	5.0	
600	33	do.....	7.0	1.0	3.0	2.0	4.0	.....	.....	.....	.....	.....	.....	.....	3.4	
43	100	Wheat.....	6.0	2.0	1.0	3.0	1.0	1.0	1.0	.0	.2	.2	.2	1.0	1.4	1.0
128	40	do.....	.2	.5	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.3	
103	18	do.....	.0	.5	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	
400	40	do.....	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	
42	60	do.....	1.0	2.0	1.0	2.0	3.0	2.0	.5	.2	.2	.5	.....	.....	1.2	
47	75	do.....	.2	.5	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.3	
123	50	do.....	2.0	1.0	.2	.2	1.0	.....	.....	.....	.....	.....	.....	.....	.9	
674	60	do.....	2.0	1.0	3.0	4.0	3.0	2.0	3.0	1.0	.....	.....	.....	.....	2.4	
496	80	do.....	2.0	2.0	1.0	1.0	3.0	1.0	.....	.....	.....	.....	.....	.....	1.7	1.7
127	90	Oats.....	.5	.2	.5	.5	2.0	5.0	4.0	2.0	5.0	4.0	4.0	2.0	2.5	
125	20	do.....	.2	.2	.0	.2	.2	.....	.....	.....	.....	.....	.....	.....	.2	
190	60	do.....	.5	.2	2.0	.2	1.0	.5	.....	.....	.....	.....	.....	.....	.7	
607	35	do.....	3.0	3.0	4.0	4.0	3.0	3.0	.....	.....	.....	.....	.....	.....	3.3	.2
400	40	Timothy...	.2	.2	.2	.2	.....	.....	.....	.....	.....	.....	.....	.....	.2	

While the wheat-scab infection during this season was generally light, there was enough to show a striking difference in the percentage of scab resulting where wheat followed corn and where wheat followed some other crop. In every field which had produced a corn crop during the previous year the percentage of wheat scab was much higher than where some other crop had preceded wheat, the average of scab infection being more than 170 per cent higher than in any other crop sequence.

The seasonal difference in the percentage of scab was due undoubtedly to weather conditions during the flowering and maturing of the wheat crop. It has been previously observed by Schmitz (18), Mortensen (16), Beckwith (2), Schaffnit (17), and others, that there is a greater abundance of wheat scab during moist seasons. Indications are that moisture was the chief factor in producing the seasonal differences in infection during these two years, as the weather during and following flowering in 1919 was very moist, whereas in 1920 the entire period was dry. Schaffnit (17), working with *Fusarium* organisms isolated from scabbed cereals, has shown that a relative humidity of not less than 55 to 60 per cent is necessary for their growth and that the substratum must contain 20 to 30 per cent of moisture.

#### STUDIES IN 1921

In 1921, an effort was made to secure data under even more uniform conditions than in the previous years. After considerable scouting, four

pairs of fields were located, each pair consisting of two adjacent wheat fields, on the same type of soil of about the same fertility, with similar topography and drainage, and sown at nearly the same time with Turkey wheat. One field of each pair had been planted to corn the previous year and the other had been cropped with either wheat or oats. Numerous counts were made in each field, as shown in Table III. Some variation in the percentage of scabbed heads occurred in each field. General observations in previous years indicated that scab infection generally was most severe at the lower field elevations. This was probably caused by the more succulent growth in these locations. Climatic conditions in 1921 were such that the growth in the low areas was not very succulent. On the contrary, the crop was thin and poorly developed in many of these lower places. An attempt was made to cover all of the different conditions in each field so as to establish correct averages. In every case the land was only gently rolling and the differences in elevation were not great. Table III shows that in this year the percentage of wheat scab was only slightly affected by differences in elevation, the greater infection, if any, occurring on the higher ground where, in this season, the wheat had made the best growth.

TABLE III.—Percentages of scab in Turkey wheat at different elevations in four pairs of adjacent fields (A, B, C, D), on brown silt loam with similar fertility, drainage, and topography, but having had different previous crops, in McLean County, Ill., in 1921

Group.	Field number.	Size of field.	Pre-vious crop.	Eleva-tion.	Percentage scab at different locations in fields.										Field average.
					1	2	3	4	5	6	7	8	Average.		
A	424	40	Corn...	High.....	40	31	43	43	.....	.....	.....	.....	39.3	.....	
				Medium....	53	52	14	26	39	41	.....	.....	37.5	.....	
				Low.....	29	26	9	19	15	25	.....	.....	20.5	31.6	
	428	60	Wheat	High.....	4	5	2	5	3	1	5	6	3.9	.....	
				Medium....	3	4	3	7	.....	.....	.....	.....	4.3	.....	
				Low.....	4	4	2	2	3	6	.....	.....	3.5	3.8	
B	610	20	Corn...	High.....	9	16	13	26	.....	.....	.....	.....	16.0	.....	
				Medium....	19	16	14	10	.....	.....	.....	.....	14.8	.....	
				Low.....	13	13	8	7	.....	.....	.....	.....	10.3	13.7	
	611	20	Wheat	High.....	1	4	4	4	.....	.....	.....	.....	3.3	.....	
				Medium....	4	5	2	4	.....	.....	.....	.....	3.8	.....	
				Low.....	2	6	3	4	.....	.....	.....	.....	3.8	3.6	
C	133	80	Corn...	High.....	22	14	12	18	.....	.....	.....	.....	16.5	.....	
				Medium....	22	22	14	12	18	14	.....	.....	17.0	.....	
				Low.....	6	8	10	12	16	14	.....	.....	11.0	14.6	
	132	80	Oats...	High.....	8	11	8	.....	.....	.....	.....	.....	9.0	.....	
				Medium....	3	6	8	.....	.....	.....	.....	.....	5.7	.....	
				Low.....	6	6	8	4	.....	.....	.....	.....	6.0	6.8	
D	109	20	Corn...	Medium....	8	4	22	18	9	6	7	11	10.6	.....	
				Low.....	11	13	5	3	.....	.....	.....	.....	8.0	9.8	
	107	40	Oats...	High.....	3	5	3	3	.....	.....	.....	.....	3.5	.....	
				Medium....	3	4	5	4	.....	.....	.....	.....	4.0	.....	
				Low.....	5	5	1	5	.....	.....	.....	.....	4.0	3.8	

In group A the wheat was drilled in a field on which the corn was still standing. This left all the old cornstalks in the wheat field. In the other groups, the corn had been removed for silage, leaving only the corn stubble and a very few broken or scattered stalks that were not picked up.

Table III shows that, as in the previous year, scab infections were more than a hundred per cent greater where wheat followed corn than where wheat followed other crops. It also shows that infection is much worse when all the cornstalks are left on the field than when nearly all of them are removed. It must be borne in mind, however, that the corn stubbles were left exposed and that they were thus an important factor in increasing the disease in fields 610, 133, and 109, as compared with fields 611, 132, and 107, respectively.

The study of the influence of the previous crop on the development of wheat scab had shown that the abundant development of perithecia on the old cornstalks determined to a large measure the relative amount of wheat scab. It was important, therefore, to determine the extent to which these perithecia would develop on stalks of relatively resistant strains of corn. Such strains of corn were available, as considerable work had been done by J. R. Holbert and his associates (10, 12<sup>4</sup>) in the breeding and selection of strains resistant to the root and stalk rots. In the fall of 1920 an experiment was planned with this corn to determine the extent to which the perithecia of *Gibberella* would develop on stalks from corn showing considerable resistance to the root- and stalk-rots. Stalks were obtained from three different selections: (1) The most disease-resistant obtainable; (2) comparatively disease-resistant; and (3) disease-susceptible. A large bundle of each kind was taken to a wheat field in the fall after the wheat had been sown and each bundle was scattered sufficiently to insure that each stalk came in contact with the soil. Just prior to wheat harvest the following year the cornstalks were collected and taken to the laboratory to determine the relative abundance of *Gibberella* perithecia on those from each selection. Estimates were made on representative portions from each stalk, each portion including a node and an internode. To establish standards, five pieces were selected with perithecia ranging in abundance from few to very numerous. On the piece where the perithecia were very numerous they covered about half the surface. Therefore, this sample was classed as 50 per cent covered. The piece having only a few perithecia was rated as 10 per cent, as this was about the proportion of surface covered. The other three portions were intermediate, differing by about 10 per cent, thus making the series 10, 20, 30, 40, and 50 per cent covered. These samples were used as standards in estimating the percentages of surface covered on all the portions of each stalk. These percentages were then averaged to get the average percentage for each stalk. The results are given in Table IV.

It will be noticed that there were not so many perithecia on stalks of the resistant selections. Nevertheless there were sufficient perithecia even on these to cause an epidemic of wheat scab under favorable conditions. These corn plants were resistant to fungus invasion during their active growing and maturing period, but after maturity and death of the tissues any corn plant is readily invaded by many soil-borne, facultative parasites, and among these *Gibberella saubinetii* is very common. It seems evident, therefore, that growing only disease-resistant corn will not aid materially in avoiding higher percentages of scab infection when wheat follows a crop of corn.

<sup>4</sup>HOLBERT, J. R., and others. PHYSICAL CHARACTERS OF THE CORN EAR IN RELATION TO DISEASE RESISTANCE AND YIELD. III. Agr. Exp. Sta. (In press.)



TABLE IV.—Percentages of stalk surface covered with perithecia of *Gibberella saubinetii* on old stalks of Yellow Dent corn in strains highly resistant, comparatively resistant, and susceptible to corn rootrot. Data obtained at Bloomington, Ill., in 1921

Stalk No.	Percentages of surface covered with <i>Gibberella perithecia</i> .		
	Most resistant selection.	Comparatively resistant.	Susceptible.
1.....	6.2	5.0	14.5
2.....	3.7	2.8	9.5
3.....	2.0	.7	19.2
4.....	0	1.7	6.7
5.....	7.5	3.3	11.5
6.....	6.5	4.3	5.0
7.....	4.4	5.7	5.5
8.....	2.2	3.5	5.7
9.....	2.8	5.8	8.6
10.....	4.4	4.4	4.4
11.....	1.7	2.9	4.5
12.....	4.1	7.0	13.2
13.....	0	2.5	11.0
14.....	1.5	15.0	5.5
15.....	2.5	1.0	8.6
16.....		3.9	13.5
17.....		1.5	10.5
18.....		4.5	6.0
19.....		2.2	5.2
20.....		10.9	5.7
21.....		2.5	12.2
Average.....	3.3	4.3	8.9

## SUMMARY OF THE THREE YEARS' DATA

The three years' data as summarized in Table V, and presented graphically in Figure 1, give the average percentages of scab under three different seasonal conditions, that is, a high amount of scab in 1919, a low amount in 1920, and a medium amount in 1921. These seasonal differences are undoubtedly due to weather conditions and can not be controlled, but the relative abundance of scab each year, whether large or small, varies with the previous cropping, and is within the control of the farmer. Whether the average percentage of infection from season to season is large or small, the farmer who sows wheat after corn can easily reduce the infection 50 per cent by using a different crop sequence in his rotations.

TABLE V.—Average percentages of scab on wheat following various crops in seven States in 1919, and in McLean County, Ill., in 1920 and 1921, being a summary of Tables I, III, and IV

Year.	Average percentage of wheat-scab infection after previous crop of—					
	Corn.	Wheat.	Rye.	Oats.	Clover.	Timothy.
1919.....	43.3	29.2	27.0	22.7	11.4	8.3
1920.....	4.6	1.0		1.7		.2
1921.....	17.4	3.7		5.3		

Corn refuse left from a previous crop generally bears an abundance of perithecia of *Gibberella saubinetii*. Morphological examination and cross inoculations have proved that this is the same organism as that found on scabbed wheat heads. Even though the cornstalks are removed from a field, the remaining corn stubble may show a large number of perithecia (Pl. 1, B, C), unless they are turned under in plowing, and it is very difficult to turn them all under (Pl. 1, A). *Gibberella saubinetii* perithecia also have been found on old straw of wheat, rye, and oats left on the field (Pl. 1, D). However, not so much infection arises from the ascospores from perithecia on wheat, rye, and oat straw because usually the fields are plowed after these crops are grown and

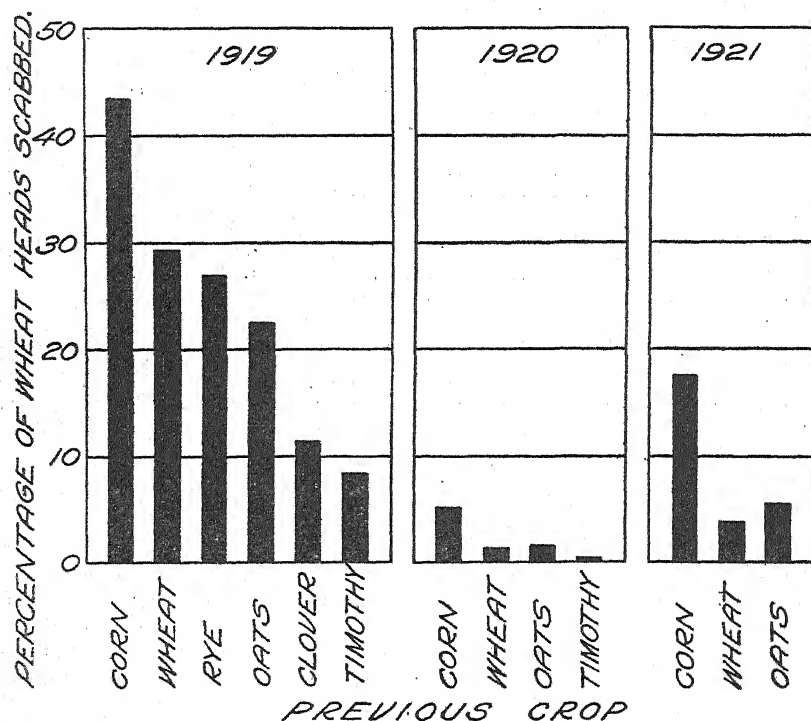


FIG. 1.—Graph showing average percentages of wheat scab following various crops in 1919, 1920, and 1921. Data summarized in Table V.

as a result most of the refuse is turned under. Although the greatest amount of initial infection probably is caused by overwintering ascospores, or conidia formed by the ascospores germinating on the moist soil and crop refuse, the mycelium of the organism also may remain viable on crop refuse and produce abundant conidia during the following spring and summer. Corn refuse bears the greatest amount of perithecia because it apparently is a better medium for growth and holds the moisture better. Therefore, it is of utmost importance to cut the cornstalks close to the ground and remove them from the field if wheat is to be seeded on the land. If winter wheat must be sown after corn, usually it is not practicable to plow the ground; but, in winter-wheat sections especially, if spring wheat is to be sown, such land should be fall plowed in order to bury, as far as possible, all corn refuse. In all

cases where practicable, however, sowing wheat after corn should be avoided.

The data discussed pertain solely to scab or head blight, as only this phase of the disease can be seen and accurately estimated. No account has been taken of seedling blight and weak plants due to the parasite in the soil attacking wheat seedlings. Investigations by Dickson (4) and Johnson and Dickson (13) show the importance of seedling blight due to soil infestation with *Gibberella saubinetii*. Therefore, the economic importance of not bringing corn and wheat together in a rotation is much greater than data on wheat scab (head blight) alone would indicate. However, it is nearly impossible to get an accurate estimate on seedling blight in a commercial field, and, therefore, this phase has not been considered in this paper.

#### CORN ROOTROT IN RELATION TO WHEAT-SCAB

The losses resulting from corn rootrot caused by *Gibberella saubinetii* can not be estimated as easily as can those resulting from wheat scab. It can be done, however, more advantageously on carefully controlled experimental plats than by an extensive survey of commercial fields. Fortunately, corn disease investigations have progressed sufficiently (12, 5) so that small quantities of corn that are highly resistant to rootrot under central Illinois conditions can be obtained. This resistant corn was used as a check on the ordinary susceptible corn in all the experiments reported below.

Comparative yields were considered the best index to the extent of rootrot. Extensive data by Holbert et al. (12, 5) show that yields are inversely correlated with the amount of disease found on the germinator and with corn rot produced by pure-culture inoculations. The latter is also shown in Table X. The disease-resistant and disease-susceptible selections, between which comparisons were made, were both selected from the same strain of Yellow Dent corn according to the methods described by Holbert et al.<sup>5</sup> The yields of two fields planted with the same kind of corn but with different previous crops can not be compared directly because there doubtless would be a difference in soil fertility. But by comparing the yield from disease-susceptible corn with the yield from disease-resistant corn, in different crop sequences, a fairly accurate determination can be made. The experimental series reported below were conducted on adjacent fields in each case, and on similar soil of the same elevation and drainage.

In 1920 an experiment was conducted to compare (1) the yields of corn grown on plats which had been cropped to clover during the two previous years and to oats in 1917 and (2) the yields from adjacent plats which had a crop of badly scabbed spring wheat on them in 1919, corn in 1918, and oats in 1917. A diagram of the plats is given in figure 2. Each plat was 4 rows wide and 10 hills long. The hills were 42 inches apart each way and were planted by hand at the rate of 3 kernels per hill. The data as given in Tables VI and VII were obtained by omitting the outside hills of each plat. Thus each plat contained 2 rows of 8 hills each, on which data were taken, a total of 16 hills with a perfect stand of 48 plants.

As shown in Tables VI and VII, the yields from disease-resistant corn were very different on the two series of plats with different previous

<sup>5</sup> HOLBERT, J. R., and others. OP. CIT.

crop successions. There was a reduction in yield of 11.6 bushels, or 13.9 per cent, on the plats where corn followed scabbed wheat. Doubtless this was due largely to differences in fertility; but no doubt a small part was due also to seedling blight and corn rootrot from the previous crops, since the resistant corn was not immune, as is shown by the inoculation experiments with pure cultures on virgin soil, the results of which are given in Table X. The yields from disease-susceptible corn, on the other hand, showed much greater variation in yield due to the previous crop. There was a reduction of 18.5 bushels, or 25.4 per cent, on the

CLEAN SOIL CLOVER 1919-1918										INFESTED SOIL SCABBED WHEAT 1919, CORN 1918									
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10

FIG. 2.—Diagram of experimental plats in 1920, data on which are presented in Tables VI and VII. Plats A, C, and E, disease-resistant corn; B, D, and F, disease-susceptible corn.

plats previously cropped with wheat. All evidence indicates that the difference between the 13.9 per cent reduction in yield of resistant corn and the 25.4 per cent reduction of susceptible corn was caused by disease-producing organisms in the soil which produced seedling blight, thus reducing the stand, as well as reducing the yield of the remaining plants. In reality, the loss from disease probably was even a little larger than this, because, as already mentioned, part of the 13.9 per cent reduction in yield from the resistant corn was most likely also caused by disease.

The experimental series of 1921, the results of which are given in Tables VIII and IX, were conducted on virgin bluegrass sod and on an

CLEAN SOIL VIRGIN BLUEGRASS SOIL PREVIOUS TO 1921									INFESTED SOIL SCABBED WHEAT 1920, SCABBED OATS 1919								
F1	F2	F3	F4	F5	F6	F7	F8	F9	F1	F2	F3	F4	F5	F6	F7	F8	F9
E1	E2	E3	E4	E5	E6	E7	E8	E9	E1	E2	E3	E4	E5	E6	E7	E8	E9
D1	D2	D3	D4	D5	D6	D7	D8	D9	D1	D2	D3	D4	D5	D6	D7	D8	D9
C1	C2	C3	C4	C5	C6	C7	C8	C9	C1	C2	C3	C4	C5	C6	C7	C8	C9
B1	B2	B3	B4	B5	B6	B7	B8	B9	B1	B2	B3	B4	B5	B6	B7	B8	B9
A1	A2	A3	A4	A5	A6	A7	A8	A9	A1	A2	A3	A4	A5	A6	A7	A8	A9

FIG. 3.—Diagram of experimental plats in 1921, data on which are presented in Tables VIII and IX. Plats A, C, and E, disease-resistant corn; B, D, and F, disease-susceptible corn; intervening and marginal plats all planted with nearly disease-free seed corn of the same variety.

adjacent field that had grown a crop of moderately scabbed winter wheat during 1920, badly scabbed oats in 1919, and corn during the previous 6 years. Bone meal had been applied to this field so that the productiveness was nearly equal to that of the virgin soil. The plats, figure 3, were of the same size and planted in the same way as those in 1920, but they were arranged differently. Between each two plats used in this experiment was a plat of corn of the same size grown from nearly disease-free seed. Thus the disease-resistant and disease-susceptible corn were bordered by the same kind of corn and apparently should have had the same opportunities. In this series all of the 40 hills in each plat were included in the stand count.

vested. The acre yield of the disease-resistant corn was 2.7 bushels, or 4.8 per cent, less on the field in which the previous crops of wheat, oats, and corn had been grown, than on the virgin sod, while the acre yield of the disease-susceptible corn was 9.4 bushels, or 17.8 per cent, less. The difference in yield of 13 per cent, then, was due very likely to disease-producing organisms in the soil. But here again, as previously explained, the actual difference probably was a little larger on account of the fact that some reduction in yield of the resistant corn also was chargeable to the attacks of *Gibberella saubinetii*. These data clearly indicate that ordinary susceptible dent corn may suffer a significant reduction in yield when grown after a crop of badly scabbed wheat.

#### CORN INOCULATIONS WITH GIBBERELLA SAUBINETII

##### THE ORGANISM

During the season of 1919, over 2,000 scabbed wheat heads collected in the survey in the six States included in the rotation studies mentioned, as well as from seven other States, were sent to the laboratory at Madison, Wis., for identification of the organisms. As reported by Dickson, Johann, and Wineland (5), 94 per cent of the scab was found to be caused by *Gibberella saubinetii*, while the remaining 6 per cent was caused principally by *Fusarium culmorum* var. *leteius* Sher. and *Fusarium avenaceum* (Fr.) Sacc. These latter came principally from northern Wisconsin and Minnesota where corn is not generally grown. During that season no perithecia of *Gibberella saubinetii* could be found on any of the corn refuse in these northern sections, while in the Corn Belt, perithecia of this fungus were very abundant. In 1920, a large number of scabbed wheat heads were collected from the States included in the Corn Belt and sent to Madison. During this season 98 per cent of the scab was found to be caused by *Gibberella saubinetii*, while most of the remaining 2 per cent was caused by *Fusarium avenaceum*.

The isolation studies would indicate, therefore, that *Gibberella saubinetii* is the principal organism concerned. In fact, it probably is the only one of economic importance in the production of wheat scab in the United States and therefore has been used in all of the inoculation experiments.

Inoculation experiments with corn growing under field conditions have been conducted for a number of years at Bloomington, Ill. It has been reported by Holbert, Dickson, and Biggar (9) that germination was lowered, early growth retarded, relative vigor throughout the season reduced, and grain production lessened by inoculation with *Gibberella saubinetii*. Since that report, inoculation experiments have been conducted on a larger scale and methods of technique have been improved. Some of the later results are given by Holbert et al. (12<sup>6</sup>). The fundamental facts first reported (9) have held true. Summarized data on some of the largest and most significant experiments will be given here.

##### METHODS

Experiments<sup>7</sup> were conducted in 1921 at Bloomington, Ill., with a strain of *Gibberella saubinetii* isolated from wheat kernels. The inoculations were made with 3-day-old cultures grown on potato dextrose agar

<sup>6</sup> HOLBERT, J. R., and others. OP. CIT.

at 20° to 22° C. The seed was placed in an aqueous spore suspension of this organism for 10 minutes just previous to planting. The concentration of this spore suspension was such that when a drop was placed on a glass slide and covered with a cover glass, about 100 spores could be seen in one field of the microscope, using a 4 mm. objective.

Two strains of corn were used, both originally from Yellow Dent. One represented the most disease-resistant strain obtainable at the time and the other represented good, average seed corn that had had no special selection toward disease resistance. These selections were similar to those used in the experiments reported in Tables VI, VII, VIII, and IX.

TABLE VI.—Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on fields previously cropped to clover and scabbed wheat, respectively, at Bloomington, Ill., in 1920

PREVIOUS CROP—CLOVER

Plat No.	Resistant seed.			Plat No.	Susceptible seed.		
	Field stand.		Acre yield.		Field stand.		Acre yield.
	Number of stalks.	Per cent.			Number of stalks.	Per cent.	
			<i>Bushels.</i>				<i>Bushels.</i>
A 1.....	37	77.1	80.9	B 1.....	34	70.8	69.6
A 2.....	42	87.5	88.9	B 2.....	39	81.3	75.8
A 3.....	43	89.6	92.8	B 3.....	41	85.4	72.0
A 4.....	39	81.3	88.9	B 4.....	41	85.4	81.3
A 5.....	40	83.3	78.6	B 5.....	43	89.6	72.7
A 6.....	37	77.1	84.2	B 6.....	39	81.3	71.2
A 7.....	43	89.6	88.9	B 7.....	41	85.4	72.7
A 8.....	40	83.3	77.0	B 8.....	39	81.3	72.7
A 9.....	39	81.3	88.8	B 9.....	42	87.5	85.2
A 10.....	43	89.6	91.2	B 10.....	39	81.3	67.3
Average ..	40.3	84.0	86.0	Average ..	39.8	82.9	74.1
C 1.....	48	100.0	79.3	D 1.....	40	83.3	68.0
C 2.....	45	93.8	80.2	D 2.....	40	83.3	78.8
C 3.....	39	81.3	78.6	D 3.....	41	85.4	67.3
C 4.....	45	93.8	94.4	D 4.....	34	70.8	69.6
C 5.....	40	83.3	81.0	D 5.....	40	83.3	62.6
C 6.....	45	93.8	78.8	D 6.....	36	75.0	63.4
C 7.....	40	83.3	77.8	D 7.....	38	79.2	73.6
C 8.....	41	85.4	90.4	D 8.....	41	85.4	80.6
C 9.....	41	85.4	80.1	D 9.....	40	83.3	76.7
C 10.....	42	87.5	81.7	D 10.....	40	83.3	69.6
Average ..	42.6	88.8	82.2	Average ..	39.0	81.3	71.0
E 1.....	43	89.6	72.0	F 1.....	36	75.0	69.3
E 2.....	46	95.8	80.4	F 2.....	42	87.5	73.6
E 3.....	41	85.4	84.0	F 3.....	45	93.8	85.7
E 4.....	43	89.6	88.7	F 4.....	39	81.3	70.8
E 5.....	44	91.7	82.9	F 5.....	40	83.3	70.8
E 6.....	40	83.3	77.2	F 6.....	39	81.3	70.0
E 7.....	38	79.2	83.6	F 7.....	39	81.3	71.2
E 8.....	39	81.3	84.3	F 8.....	37	77.1	72.8
E 9.....	40	83.3	87.4	F 9.....	35	72.9	70.4
E 10.....	41	85.4	88.1	F 10.....	40	83.3	79.9
Average ..	41.5	86.5	82.9	Average ..	39.2	81.7	73.5
General average.	41.5	86.4	83.7	General average.	39.3	81.9	72.9

TABLE VI.—Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on fields previously cropped to clover and scabbed wheat, respectively, at Bloomington, Ill., in 1920—Continued

## PREVIOUS CROP—SCABBED WHEAT

Resistant seed.				Susceptible seed.			
Plat No.	Field stand.		Acre yield.	Plat No.	Field stand.		Acre yield.
	Number of stalks.	Per cent.			Number of stalks.	Per cent.	
			<i>Bushels.</i>				<i>Bushels.</i>
A 1.....	39	81.3	65.6	B 1.....	34	70.8	54.5
A 2.....	45	93.8	73.5	B 2.....	37	77.1	49.5
A 3.....	46	95.8	81.4	B 3.....	37	77.1	43.6
A 4.....	41	85.4	77.0	B 4.....	34	70.8	55.1
A 5.....	38	79.2	76.2	B 5.....	34	70.8	61.0
A 6.....	43	89.6	80.1	B 6.....	44	91.7	66.5
Average..	42.0	87.5	75.6	Average..	36.7	76.4	55.0
C 1.....	42	87.5	67.6	D 1.....	40	83.3	63.9
C 2.....	44	91.7	56.0	D 2.....	38	79.2	62.0
C 3.....	39	81.3	68.7	D 3.....	42	87.5	45.1
C 4.....	38	79.2	73.0	D 4.....	44	91.7	50.8
C 5.....	41	85.4	74.5	D 5.....	37	77.1	59.4
C 6.....	43	89.6	69.8	D 6.....	39	81.3	53.2
Average..	41.2	85.8	68.3	Average..	40.0	83.3	55.7
E 1.....	42	87.5	76.8	F 1.....	36	75.0	47.6
E 2.....	46	95.8	73.3	F 2.....	41	85.4	51.8
E 3.....	45	93.8	74.7	F 3.....	45	93.8	55.1
E 4.....	40	83.3	66.8	F 4.....	43	89.6	50.4
E 5.....	47	97.9	74.4	F 5.....	43	89.6	52.2
E 6.....	42	87.5	69.1	F 6.....	39	81.3	58.2
Average..	43.7	91.0	72.5	Average..	41.2	85.8	52.6
General average.	42.3	88.1	72.1	General average.	39.3	81.8	54.4

TABLE VII.—Summary of data given in Table VI on the relation of previous cropping to the yield of corn grown from disease-resistant and disease-susceptible seed at Bloomington, Ill., in 1920

Previous crop.	Acre yield of corn grown from disease-resistant seed.	Acre yield of corn grown from disease-susceptible seed.
Clover (relatively clean soil).....	<i>Bushels.</i> 83.7 ± 0.8	<i>Bushels.</i> 72.9 ± 0.8
Scabby wheat (infested soil).....	72.1 ± 0.9	54.4 ± 1.1
Difference.....	11.6 ± 1.2	18.5 ± 1.4

TABLE VIII.—Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on a field of newly broken bluegrass sod and on a field previously cropped with scabbed wheat, at Bloomington, Ill., in 1921

## PREVIOUS CROP—BLUEGRASS SOD

Plat No.	Resistant seed.			Plat No.	Susceptible seed.		
	Field stand.		Acre yield.		Field stand.		Acre yield.
	Number of stalks.	Per cent.			Number of stalks.	Per cent.	
			<i>Bushels.</i>				<i>Bushels.</i>
A 1.....	114	95.0	65.7	B 1.....	115	95.8	56.3
A 2.....	114	95.0	61.3	B 2.....	110	91.7	51.1
A 3.....	113	94.2	57.0	B 3.....	108	90.0	45.2
A 4.....	119	99.2	59.6	B 4.....	110	91.7	51.9
A 5.....	101	84.2	50.8	B 5.....	108	90.0	47.8
A 6.....	105	87.5	48.7	B 6.....	110	91.7	54.5
A 7.....	100	83.3	46.0	B 7.....	103	85.8	51.3
A 8.....	107	89.2	54.3	B 8.....	106	88.3	47.4
A 9.....	114	95.0	62.4	B 9.....	115	95.8	46.0
Average..	109.7	91.4	56.2	Average..	109.4	91.2	50.2
C 1.....	117	97.5	60.9	D 1.....	117	97.5	52.4
C 2.....	116	96.7	49.8	D 2.....	114	95.0	58.5
C 3.....	119	99.2	60.7	D 3.....	116	96.7	52.8
C 4.....	119	99.2	56.9	D 4.....	115	95.8	48.4
C 5.....	120	100.0	48.0	D 5.....	116	96.7	49.8
C 6.....	115	95.8	52.2	D 6.....	116	96.7	48.2
C 7.....	119	99.2	53.5	D 7.....	117	97.5	41.4
C 8.....	116	96.7	51.9	D 8.....	109	90.8	52.1
C 9.....	120	100.0	58.7	D 9.....	116	96.7	53.7
Average..	117.9	98.3	54.7	Average..	115.1	95.9	50.8
E 1.....	117	97.5	67.0	F 1.....	108	90.0	66.8
E 2.....	119	99.2	67.4	F 2.....	86	71.7	46.1
E 3.....	119	99.2	67.7	F 3.....	109	90.8	63.4
E 4.....	118	98.3	51.3	F 4.....	104	86.7	52.6
E 5.....	117	97.5	60.0	F 5.....	100	83.3	47.6
E 6.....	116	96.7	56.3	F 6.....	105	87.5	58.7
E 7.....	114	95.0	59.6	F 7.....	107	89.2	54.6
E 8.....	110	91.7	58.5	F 8.....	101	84.2	66.3
E 9.....	116	96.7	45.0	F 9.....	109	90.8	61.3
Average..	116.2	96.9	59.2	Average..	103.2	86.0	57.5
General average.	114.6	95.5	56.7	General average.	109.2	91.0	52.8



TABLE VIII.—Data showing field stands and acre yields from disease-resistant and disease-susceptible strains of Yellow Dent corn, both grown on a field of newly broken bluegrass sod and on a field previously cropped with scabbed wheat, at Bloomington, Ill., in 1921—Continued

## PREVIOUS CROP—SCABBED WHEAT

Resistant seed.				Susceptible seed.			
Plat No.	Field stand.		Acre yield.	Plat No.	Field stand.		Acre yield.
	Number of stalks.	Per cent.			Number of stalks.	Per cent.	
			<i>Bushels.</i>				<i>Bushels.</i>
A 1.....	114	95.0	52.4	B 1.....	104	86.7	48.4
A 2.....	119	99.2	59.3	B 2.....	106	88.3	50.9
A 3.....	114	95.0	48.9	B 3.....	113	94.2	38.8
A 4.....	115	95.8	46.4	B 4.....	112	93.3	30.6
A 5.....	114	95.0	51.3	B 5.....	114	95.0	31.6
A 6.....	113	94.2	50.8	B 6.....	110	91.7	37.0
A 7.....	116	96.7	61.7	B 7.....	109	90.8	50.6
A 8.....	112	93.3	58.7	B 8.....	111	92.5	43.7
A 9.....	115	95.8	50.2	B 9.....	111.	92.5	36.6
Average..	114.7	95.6	53.3	Average..	110.0	91.7	40.9
C 1.....	115	95.8	67.3	D 1.....	115	95.8	49.5
C 2.....	116	96.7	55.0	D 2.....	102	85.0	47.3
C 3.....	112	93.3	51.0	D 3.....	98	81.7	42.8
C 4.....	119	99.2	50.2	D 4.....	108	90.0	39.5
C 5.....	114	95.0	55.4	D 5.....	113	94.2	41.2
C 6.....	117	97.5	50.4	D 6.....	112	93.3	45.6
C 7.....	119	99.2	57.0	D 7.....	116	96.7	40.1
C 8.....	115	95.8	51.3	D 8.....	114	95.0	48.2
C 9.....	120	100.0	46.0	D 9.....	115	95.8	43.2
Average..	116.3	96.9	53.7	Average..	110.3	91.9	44.2
E 1.....	118	98.3	60.2	F 1.....	99	82.5	52.6
E 2.....	118	98.3	66.6	F 2.....	105	87.5	49.7
E 3.....	117	97.5	46.1	F 3.....	106	88.3	44.9
E 4.....	114	95.0	49.7	F 4.....	106	88.3	51.1
E 5.....	114	95.0	57.8	F 5.....	105	87.5	43.9
E 6.....	118	98.3	54.3	F 6.....	106	88.3	36.2
E 7.....	113	94.2	59.3	F 7.....	95	79.2	41.0
E 8.....	111	92.5	50.4	F 8.....	95	79.2	45.2
E 9.....	116	96.7	49.6	F 9.....	109	90.8	42.3
Average..	115.4	96.2	54.9	Average..	102.9	85.7	45.2
General average.	115.5	96.2	54.0	General average.	107.7	89.8	43.4

TABLE IX.—Summary of data given in Table VIII on the relation of previous cropping to the yield of corn grown from disease-resistant and disease-susceptible seed at Bloomington, Ill., in 1921

Previous crop.	Acre yield of corn grown from disease-resistant seed.	Acre yield of corn grown from disease-susceptible seed.
	<i>Bushels.</i>	<i>Bushels.</i>
Virgin bluegrass sod (clean soil).....	56. 7±1. 0	52. 8±0. 7
Scabbed wheat (infested soil).....	54. 0±1. 2	43. 4±1. 0
Difference .....	2. 7±1. 6	9. 4±1. 2

The plats were located on a uniform piece of virgin, bluegrass sod with good drainage. The size of these plats was 4 rows wide by 10 hills long. Alternate with every plat planted with inoculated seed was a plat planted with uninoculated seed. As shown in Table X, these plats were replicated a number of times for both the disease-resistant and disease-susceptible selections. All the hills were 42 inches apart each way and were planted at the rate of 3 kernels per hill. As in all the other corn experiments, the plantings were made by hand to insure accuracy. Field stand and yield were taken on the entire plat 4 rows wide by 10 hills long, a total of 40 hills with a perfect stand of 120 plants.

#### RESULTS

The field stand, the number of vigorous plants, and the acre yield were reduced in every instance by the inoculations, as shown in Table X. The reductions in yield were small, averaging 3 per cent, when seed of disease-resistant corn was used. A large decrease in yield, averaging 18 per cent, resulted from inoculating seed of disease-susceptible corn, which is representative of the corn in most general commercial use. By following the recommendations given by Holbert and Hoffer (10), and Holbert et al.,<sup>8</sup> seed corn that is disease-resistant to a considerable degree can be obtained by the average farmer, and thus at least part of the loss due to this organism can be avoided. In fact, disease-resistant seed is already being used, and is gaining in favor very rapidly in many parts of the Corn Belt.

The reductions in yield from susceptible corn were greater than the reductions in stand and were more comparable to the reduction in percentage of vigorous plants. This relationship in respect to vigorous plants has been previously pointed out by Holbert et al. (12). These experiments, as well as others previously reported, show that *Gibberella saubinetii*, isolated from infected wheat, is an active parasite on corn. The organism has not been found to go up through the stalk during the active growing period, but apparently confines its attack to the underground parts of the seedling, thus decreasing stand and plant vigor. This results in reduced resistance to later invasions by other parasites and in low yields of corn of poor quality.

<sup>8</sup> HOLBERT, J. R. and others. OP. CIT.

TABLE X.—Data showing the field stands, percentages of vigorous plants, and acre yield from disease-susceptible and disease-resistant strains of Yellow Dent corn uninoculated and inoculated with *Gibberella saubinetii*, when grown on bluegrass sod at Bloomington, Ill., in 1921

Character of corn.	Seed inoculation.	Number of replications.	Number of plants.	Per cent- age of field stand.	Per cent- age of vigorous plants.	Acre yield.	Reduction in yield caused by inoculation.	
						<i>Bushels.</i>	<i>Bushels.</i>	<i>Per ct.</i>
Disease-sus- ceptible.	{Control. ....	8	888	92.5	79.6	59.1±1.1	.....	.....
	{ <i>G. saubinetii</i> ..	4	386	80.4	59.4	48.6±1.9	10.5±2.2	17.8
Disease-sus- ceptible.	{Control. ....	8	878	91.5	75.8	54.6±1.4	.....	.....
	{ <i>G. saubinetii</i> ..	4	385	80.2	58.9	44.7±1.3	9.9±1.9	18.1
Disease-re- sistant.	{Control. ....	28	3,300	98.2	90.4	74.2±0.9	.....	.....
	{ <i>G. saubinetii</i> ..	14	1,572	93.6	80.9	71.6±1.3	2.6±1.6	3.5
Disease-re- sistant.	{Control. ....	28	3,259	97.0	87.2	72.1±0.9	.....	.....
	{ <i>G. saubinetii</i> ..	14	1,556	92.6	78.2	70.4±1.1	1.7±1.4	2.4

#### CROP ROTATION FOR THE CORN BELT

It is evident that wheat should not follow corn in a rotation if wheat scab is to be held in check. Even though the stalks are removed and the field is plowed, some corn stubble will not be turned under and some will be pulled up again by the harrow. The wheat-scab infection in this case will be much less than if most of the cornstalks were left on the field, but it will be higher than it would be if there had been no corn preceding the wheat.

In a four-year rotation, including one year of corn and one year of wheat, there would be considerable advantage from the standpoint of controlling wheat scab and corn rootrots in arranging the rotation so that the wheat will neither directly precede nor follow the corn crop. Other small grains such as oats or barley, soybeans, clover or other legumes, or timothy, would be desirable to use as the intervening crops, as they are either immune or generally only slightly susceptible to the attacks of *Gibberella saubinetii*.

In large parts of the Corn Belt it may be desirable to grow corn more frequently than one year in four. In that case it seems best to use a five-year rotation with two years of corn in succession. For the other three years the same plan as above can be followed.

Some people insist on using a rotation of two years' corn in every four, with wheat as one of the intervening crops. In that case the wheat should immediately precede rather than follow the corn crop, taking care to use the best disease-resistant seed corn obtainable. It seems doubtful, however, whether a crop rotation of 50 per cent corn can be recommended from the standpoint of permanent crop improvement.

#### SUMMARY

Wheat scab was most severe where wheat followed corn in the rotation.

The yield of corn susceptible to rootrot was considerably reduced where corn followed badly scabbed wheat in the rotation.

*Gibberella saubinetii* was the principal organism isolated from scabbed wheat heads. The same organism was found very abundantly on old corn-stalks.

When used as an inoculum on disease-susceptible corn this organism caused a considerable decrease in stand, general vigor, and yield.

Resistant strains of corn, field selected, well cured, and also selected on the germinator for vigor and freedom from disease, were injured to a less extent by inoculation with the wheat-scab organism. Such corn also did not suffer much reduction in yield when grown after scabbed wheat.

A crop rotation in which the wheat crop neither directly precedes nor follows the corn crop seems highly advantageous for the corn-wheat sections.

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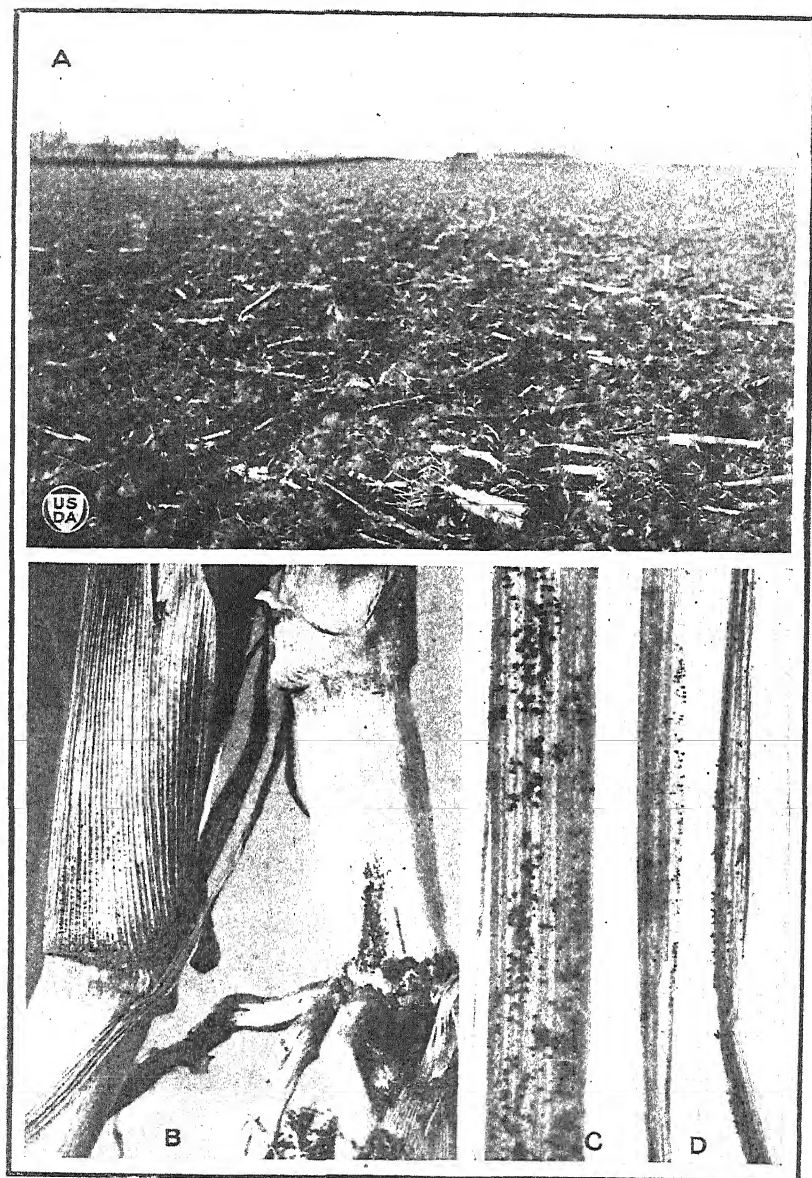
PLATE 1

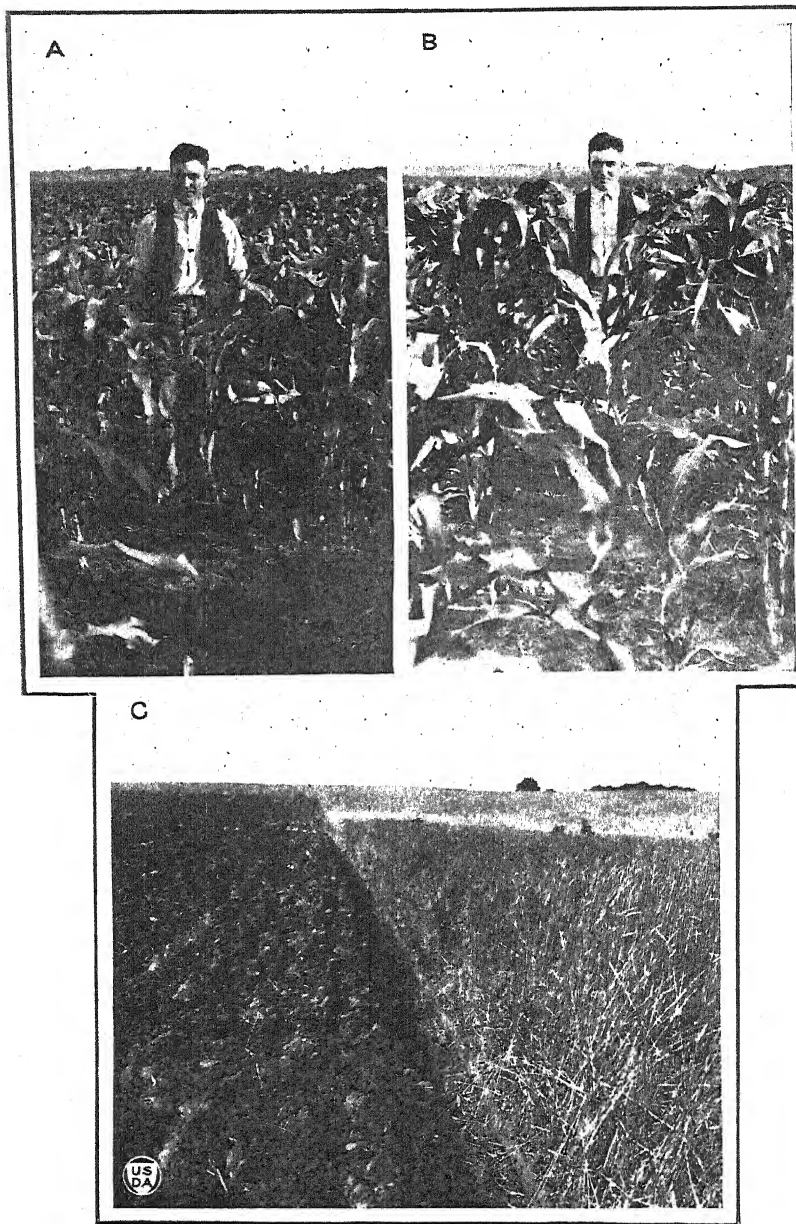
*Gibberella saubinetii* on crop refuse

A.—Winter wheat sown on corn land. The cornstalks were broken down with a drag, after which the field was disked both ways and then plowed. Enough diseased stalks remained on the surface to carry scab to the entire wheat crop if weather conditions were favorable for the development of scab during and immediately following the blossom period.

B, C.—Perithecia of *Gibberella saubinetii* on cornstalks and stubble left on the surface of the soil.

D.—Perithecia of *Gibberella saubinetii* on wheat stubble and straw which was found on the surface of the ground in the vicinity of a straw stack.







## PLATE 2

### Corn root and stalk rot caused by *Gibberella saubinetii*

A, B.—The influence of seed inoculation with *Gibberella saubinetii* on the development of corn plants. A, Yellow Dent corn planted on clean land; seed inoculated with *G. saubinetii* at time of planting. B, control. Note the difference in height of plants of the same age in inoculated and control rows.

C.—Plowing under a field of Marquis wheat ruined by scab. The yield of disease-susceptible corn planted on this field the following season was greatly reduced, whereas the disease-resistant corn gave fair yields.



# MEASUREMENT OF LINKAGE VALUES<sup>1</sup>

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The demonstration that Mendelian characters are inherited in groups that correspond to the chromosomes has been followed by much intensive work directed to the determining of the relative position of the genes in the individual chromosomes.

If two Mendelian characters are shown to be correlated in transmission their genes are assumed to lie in the same chromosome, and are said to be linked. The early idea that the linkage relations might exhibit a numerical regularity comparable to Mendelian ratios has been abandoned. There is now much evidence to show that the degree of association of genes assumed to lie in the same chromosome is profoundly influenced by environmental factors.

Since closely related progenies frequently show widely divergent cross-over ratios for the same pair of characters, a high degree of accuracy in measuring the ratio may seem to be of little value. But in all work that aims to test or to extend knowledge of the interrelations of characters it is of the greatest importance to determine whether any two characters are inherited independently or show a low degree of linkage.

When the linkage is loose a large number of individuals is required to establish the linkage. In all such cases it is necessary to be sure that the method used for demonstrating the relationship of two characters is accurate. If the method is such that a correlation is indicated where none exists, large numbers will only inspire confidence in a spurious linkage.

The most generally used method of determining the linkage relations of two characters is to cross individuals of the  $F_1$  with individuals that are homozygous for the recessive allelomorphs of both characters. The sum of the individuals representing the two crossover or nonparental combinations expressed as a percentage of the total population is then taken as representing the percentage of the gametes in which crossing-over has taken place. This is undoubtedly the most simple and direct method, for if dominance is complete each back-crossed individual indicates directly the nature of the  $F_1$  gamete that entered into its composition.

With many plants this method of back-crossing can not be used. Often the technique is difficult and it is impossible to obtain back-crossed individuals in sufficient numbers to afford reliable averages. In locating new recessive characters by the back-cross method there is the loss of a generation necessary to obtain a double recessive stock. In other instances double recessive individuals are weak and it is difficult to maintain the stock. Because of these difficulties in determining linkage values by the direct method many investigators have come to use  $F_2$  populations.

<sup>1</sup> Received for publication Feb. 1, 1924.

A number of methods have been proposed for determining the cross-over ratio from an  $F_2$  population, any of which will give accurate results for a theoretically perfect population. In practice, however, disturbing factors, such as differential death rates or indistinct classes frequently cause significant departures from a theoretically perfect population.

Disturbing factors are made manifest by departures from the expected Mendelian ratios for each character. Deviations from expected Mendelian ratios may be due to a variety of causes any of which may be without effect on the percentage of gametes in which crossingover has taken place, but which may influence the apparent percentage of crossingover as determined from zygotic ratios.

The results obtained by the different formulae are variously affected by departures from expected Mendelian ratio depending on the cause of the deviation, and there seems to be no single method that will give the most accurate results in all cases.

Although it may not be possible to determine with certainty the cause of any given departure from an expected Mendelian ratio, frequently there will be strong presumptive evidence in favor of some one cause, and when such is the case it should be of value to be able to choose a method that is not seriously affected by the disturbing factor. In the following pages the methods in most general use have been brought together and compared empirically as to their accuracy.

#### NATURE OF ABERRANT MENDELIAN RATIOS

Departures from expected Mendelian ratios may be due to irregularities in the behavior of the gametes or of the zygotes. Several types of gametic disturbances have been recognized, such as the formation of dominant and recessive gametes in unequal numbers, differential mortality, and selective fertilization. The effect on linkage relations is the same in all cases—that is, effective gametes bearing the dominant and recessive members of an allelomorphic pair are unequal in number.

Similarly, there may be a variety of ways in which one zygotic class is influenced adversely, but the general result is a differential mortality or a difference in the survival value of dominant and recessive individuals.

In addition to these two main causes of aberrations there is the slightly different case that results from mistakes in the classification of zygotes. The deviation is, of course, zygotic, but while the numbers representing one member of the character pair are increased at the expense of the other, it is not the same as a differential mortality, for if there is a linkage between the two character pairs a differential death rate in one character must necessarily affect the ratio of dominant to recessive in the other character, whereas mistakes in the classification of one character do not affect the ratio of dominant to recessive in the second character.

In discussing the various measures of linkage the following terminology will be used:

$AB, Ab, aB, ab$  = the number of individuals in each of the four zygotic classes in a hybrid involving two character pairs  $Aa$  and  $Bb$ .

$n$  = total number of individuals:

$p$  = that part of the total number of gametes in which crossingover has taken place expressed as a decimal fraction. \*

$1 - p$  = noncrossover gametes.

The percentage of crossingover is then  $p \times 100$  and the crossover ratio is  $\frac{p}{1-p} : \frac{1-p}{p}$ .

## MEASUREMENT OF LINKAGE BY THE BACK-CROSS METHOD

The back-cross method, sometimes called the direct method, may be used when the cross is of the nature  $Aa Bb \times aa bb$ . The formula is:

$$p = \frac{Ab + aB}{n}$$

The probable error of this determination is usually given as:

$$E_p = .6745 \sqrt{\frac{p(1-p)}{n}}$$

Even this approved method of determining the degree of linkage may not be entirely free from error when the Mendelian ratios do not agree with the expected.

Haldane states (5,<sup>2</sup> p. 292) that by the method of back-crossing "we automatically eliminate the effects of differential mortality due to one factor, or to both if they affect the mortality independently." This appears to be an error. Take a simple example with no linkage, if there is no differential mortality the four zygotic classes will be equal. If now in such a population 50 per cent of the recessive class of each character fails to survive, the observed classes will be in the proportion 4  $AB$ , 2  $Ab$ , 2  $aB$ , 1  $ab$ , indicating a linkage between dominants with 44 per cent crossing-over,  $4/9 = .44+$ .

If the Mendelian ratios are aberrant in only one character the linkage relations are not affected, but where there are departures in both, the per cent of crossover individuals will fail to indicate the true ratio of crossover to noncrossover gametes. If the extent of the error or the spurious linkage be designated  $e$ , then its value is  $e = .5 - r - s + 2 rs$  where  $r$  = the number of individuals recessive with respect to one character divided by the total number of individuals and  $s$  = the corresponding determination with respect to the other character.

If either  $r$  or  $s = 0.5$ ,  $e$  will equal 0.0—that is, there is no spurious linkage. If both  $r$  and  $s$  depart from 0.5 in the same direction there will be a spurious linkage between dominants. If  $r$  and  $s$  depart from 0.5 in opposite directions the spurious linkage will be between dominant and recessive.

To eliminate the effects of differential mortality on the measurements of linkage in back-crosses, Bridges (2, p. 3) proposed the method of balanced viability. The procedure is to average the results obtained from two original crosses in which the parental combinations are reversed, that is,  $AABB \times aabb$  and  $Aabb \times aaBB$ . This method practically eliminates the effects of differential mortality, either in gametes or zygotes and undoubtedly is the most desirable method to use whenever material for making the two crosses can be made available.

METHODS FOR DETERMINING LINKAGE VALUES FROM AN  $F_2$  POPULATION

Three general methods have been proposed for measuring linkage values in an  $F_2$  population. These are, Yule's coefficient of association, "Q," Emerson's method, "P," and Haldane's method "T."

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 891.

## YULE'S COEFFICIENT OF ASSOCIATION

The formula for  $Q$  is:

$$Q = \frac{Ab \times aB - AB \times ab}{Ab \times aB + AB \times ab} \dots \dots \dots (1)$$

where  $AB$ ,  $Ab$ ,  $aB$ , and  $ab$  represent the numbers of individuals in the four zygotic classes of an  $F_2$  population. The probable error of  $Q$  is:

$$E_Q = .6745 \times \frac{1-Q^2}{2} \sqrt{\frac{1}{AB} + \frac{1}{Ab} + \frac{1}{aB} + \frac{1}{ab}}$$

By reference to a table giving values of  $Q$  for the various values of  $p$ , the value of  $p$  corresponding to the observed  $Q$  is assumed to be the  $p$  of the observed population.

A short table of the values of  $Q$  for integral gametic ratios where dominants are linked was given by the author (3). It was pointed out by Bridges (1) that a different table was needed when dominant and recessive are linked and for accurate determinations it is, of course, necessary to resort to interpolations. These difficulties may be obviated by using the following formula:

$$p^2 = \frac{\sqrt{4-2Q(Q-1)} - 2}{2Q} \dots \dots \dots (2)$$

To make this formula applicable when dominants are linked it is necessary to retain the arrangement of the zygotic classes in the following order,  $\frac{(Ab \times aB) - (AB \times ab)}{(Ab \times aB) + (AB \times ab)}$ . When the linkage is between dominants and  $Q$  is negative,  $p$  will be greater than 0.5 and will represent the noncross-over group.

In a back-cross the coefficient of association also may be used to measure the degree of linkage and the result is not affected by differential death rates. In back-crosses  $p = \frac{Q-1 + \sqrt{1-Q^2}}{2Q}$ .

## EMERSON'S METHOD

The second method for evaluating  $p$  was proposed by Emerson (4). Stated in terms of  $p$  and  $1-p$  instead of the  $r$  and  $s$  of the original formula, and taking  $AB + ab$  as the crossover classes, the determination becomes

$$p = \sqrt{\frac{2(AB+ab)}{n} - 1} \dots \dots \dots (3)$$

No formula for the probable error of this method has been suggested, but the formula for the probable error of a ratio given under the back-cross method should provide a fair measure of the reliability of results. This method has the merit of extreme simplicity, but as Emerson pointed out it becomes unreliable when there are wide departures from expected Mendelian ratios. The method has been extended by Woodworth (7) to include multiple-factor characters. In such cases the use of  $p$  and  $1-p$  for the crossover and noncrossover gametes instead of  $r$  and  $s$ .

results in a very material simplification. Thus Woodworth's formula (2) for two characters segregating in the ratios 3 : 1 and 15 : 1 which is given as  $12r^2 + 11(s^2 + 2rs) : 3r^2 + 4(s^2 + 2rs) : s^2 + 2rs : r^2$ , becomes  $11 + p^2 : 4 - p^2 : 1 - p^2 : p^2$ , and the formula for evaluating  $p$  from an

observed zygotic distribution becomes,  $p = \sqrt{\frac{8(AB + ab)}{n} - 5.5}$

Formulae for calculating  $p$  for some of the zygotic distributions most frequently encountered are given below:

$$3 : 1 \text{ and } 1 : 1 \quad p = \frac{2(AB + ab)}{n} - 5.5$$

$$63 : 1 \text{ and } 3 : 1 \quad p^2 = \frac{32(AB + ab)}{n} - 23.5$$

$$15 : 1 \text{ and } 15 : 1 \quad p^2 = \frac{32(AB + ab)}{n} - 28$$

$$15 : 1 \text{ and } 3 : 1 \quad p^2 = \frac{8(AB + ab)}{n} - 5.5$$

$$9 : 7 \text{ and } 3 : 1 \quad p^2 = \frac{16(AB + ab) - 7n}{6n}$$

$$9 : 7 \text{ and } 9 : 7 \quad p^2 = \frac{32(AB + ab) - 14n}{9n}$$

$$9 : 7 \text{ and } 15 : 1 \quad p^2 = \frac{32(AB + ab) - 17n}{3n}$$

#### HALDANE'S METHOD

The method proposed by Haldane may be written in the form:

$$p = \sqrt{T} = \sqrt{\frac{3t_a t_1 + (2 + t_a)t_4}{2 + 4t_a}} \dots \dots \dots (4)$$

where

$$t_1 = \frac{4AB}{n} - 2$$

$$t_4 = \frac{4ab}{n}$$

$$t_a = \frac{t_1 + t_4}{2}$$

$t_a$  is an approximate value and when the observed classes are wide departures from expected Mendelian ratios it is necessary to make a further approximation by substituting  $T$  for  $t_a$  in formula (4).

The method used in deriving the formula is to determine the most probable value of  $p^2$  for an observed population where the departures from expected Mendelian ratios are the result of errors of sampling. The probable error is given by Haldane (5, p. 295) as:

$$E_p = .477 \sqrt{\frac{(2 + p^2)(1 - p^2)}{(1 + 2p^2)n}}$$

Haldane states (5, p. 294) that the method gives the same value for  $p$  as Yule's coefficient of association. This is, of course, true for a perfect distribution and it appears to hold also where the Mendelian ratio of only one of the characters is disturbed through a differential mortality of either gametes or zygotes, but where both characters are affected the two methods do not give identical results. Take for example a case with no linkage, that is  $p = .5$ , in which  $\frac{1}{2}$  the recessive gametes of both characters are not effective. The observed zygotic classes would be 64: 8: 8: 1,  $n = 81$ ,  $Q = 0$ , giving  $p = .5$ .

Haldane's formula gives  $p = .7111$  as the first approximation. As a result of ten successive substitutions of  $T$  for  $t_a$  the value of  $p$  is reduced to 0.6626 the last substitution producing no change in the first four decimal places. It is clear that 0.5 is not the asymptote.

#### COMPARISON OF METHODS

In determining the per cent of crossingover from an  $F_2$  population it is necessary to bear in mind that a value of  $p$  which gives a constructed population in closest agreement with the observed population may not be the correct value or the best that it is possible to find.

Before any attempt can be made to evaluate  $p$  it is necessary to make some assumption regarding the factorial composition of the characters involved. If these assumptions are wrong any formula will lead, of course, to an erroneous value of  $p$ . It follows, further, that if there are departures from the expected Mendelian ratios, whether due to chance, selective fertilization, differential death rates or mistakes in classification, these departures may introduce errors into the calculation of  $p$  when populations with perfect Mendelian ratios are used as criteria. A method which would determine  $p$  by reference to a population with the observed instead of the theoretical Mendelian ratios should be free from this defect.

Of the proposed methods the coefficient of association,  $Q$ , is the one that most nearly meets this requirement. Since  $Q$  is based on the relation that exists between the product of the two crossover classes as compared with the product of the two noncrossover classes it is unaffected by zygotic changes in Mendelian ratios.

$Q$  is slightly affected by mistakes in classification, and by differential fertilization, but the most frequent cause of departures from Mendelian ratios is a differential death rate of zygotes and this has no effect on the value of  $Q$ . That  $Q$  has decided limitations as a measure of correlation should not affect its use as a measure of linkage. There is, however, one serious drawback to the use of  $Q$ . When any one of the classes is small, changes in this class produce a disproportionately large effect on the value of  $Q$ . When one class is 0 and  $Q$  equals 1, it may be open to argument whether the correlation is or is not perfect, but certainly it does not follow that the linkage is complete.

When dominant and recessive are linked and the population is small failure to recover the double recessive class may result from errors of sampling. In such cases  $p$  may be calculated from the other three classes as follows:

$$p = \sqrt{\frac{2 AB - 2 (Ab + aB)}{2 AB + (Ab + aB)}}$$

Similarly, if there is difficulty in distinguishing any two classes as, for example,  $aB$  and  $ab$   $p$  may be calculated from the ratio of  $AB$  to  $Ab$  or  $AB$  to the total

$$p = \sqrt{\frac{AB - 2 Ab}{AB + Ab}} \text{ or } p = \sqrt{\frac{4 AB - 2 n}{n}}$$



$\chi^2$  AS A MEASURE OF LINKAGE

One of the most fundamental determinations in connection with linkage problems is to discriminate between a low linkage value and independent inheritance. This is done by calculating the  $\chi^2$  of the observed population from a theoretical population of the same size with no linkage.

If the theoretical population is constructed in accordance with the expected Mendelian ratio the result will be erroneous unless the observed population conforms exactly to the expected Mendelian ratios. Any irregularity in the behavior of the individual characters will increase  $\chi^2$  and may indicate a linkage when none exists.

It would seem that the correct procedure should be to construct the theoretical population from the observed instead of the expected Mendelian ratios.

A theoretical population,  $A'B'$ ,  $A'b'$ ,  $a'B'$  and  $a'b'$  of the same size and having the same Mendelian ratios may be constructed as follows:

$$\begin{aligned} a'b' &= \frac{(aB+ab) \times (Ab+ab)}{n} \\ a'B' &= \frac{(aB+ab) \times (AB+aB)}{n} \\ A'b' &= \frac{(AB+Ab) \times (Ab+ab)}{n} \\ A'B' &= \frac{(AB+Ab) \times (AB+aB)}{n} \end{aligned}$$

The observed distribution is compared with the theoretical distribution and  $\chi^2$  is determined as follows:

$$\chi^2 = \frac{(AB-A'B')^2}{A'B'} + \frac{(Ab-A'b')^2}{A'b'} + \frac{(aB-a'B')^2}{a'B'} + \frac{(ab-a'b')^2}{a'b'}$$

The degree of confidence to be placed in the observed departure is indicated in the following table:

$\chi^2$	Odds against the deviation being due to chance <sup>1</sup> .
1.....	.2 to 1
2.....	.7 to 1
3.....	1.6 to 1
4.....	2.8 to 1
5.....	4.8 to 1
6.....	8.0 to 1
7.....	12.9 to 1
8.....	20.7 to 1
9.....	33.1 to 1
10.....	52.9 to 1
11.....	84.3 to 1
12.....	134.4 to 1
13.....	214.7 to 1
14.....	343.2 to 1
15.....	549.4 to 1
16.....	880.8 to 1
17.....	1,413.4 to 1
18.....	2,271.7 to 1
19.....	3,662.0 to 1
20.....	5,881.4 to 1

<sup>1</sup> Calculated from the values of  $P$  given in Pearson's "Tables for Statisticians and Biometrists," Table XII (c).

Since the different methods of calculating crossover ratios are variously affected when the Mendelian ratios show departures from the expected, it may be of value to determine empirically how Mendelian deviations of the various kinds affect the results obtained by the different methods.

It would be of great interest to have the effects of Mendelian deviations on the various methods investigated by a competent mathematician, but until this is done a comparison of examples may be of some assistance.

Deviations from expected Mendelian ratios fall into three groups:

(1) Effective gametes of the recessive and dominant classes are not produced in equal numbers—Differential mortality of gametes.

(2) Individuals showing the dominant or recessive character may not survive in equal percentages—Differential mortality of zygotes.

(3) Individuals showing the dominant or recessive character may be wrongly classified as belonging to the opposite class.

In each of the above the departure may affect one or both characters.

Back-crosses and straight  $F_2$  populations will be considered separately. In the case of a back-cross (1) and (2) produce the same result.

It should be kept in mind that the differential death rates with which we are concerned and the effect of which we wish to eliminate are those in which one member of an allelomorphic pair, considered without respect of other Mendelian characters, has a higher death rate than its mate. Death rates due to incompatible gametic or zygotic combinations, may be distinguished from linkage by the method of balanced inviability proposed by Bridges.

Table I shows the effect of the various forms of Mendelian aberrations on a back-crossed population. It will be noted that in many cases a formula gives the correct result when  $p = .5$  and does not give the correct result when there is a linkage.

Table II compares the accuracy of the three methods in  $F_2$  populations where there is no linkage.

It appears that  $Q$  gives the correct result except where one of the classes is not represented.

$T$  likewise gives the correct result with either gametic or zygotic disturbances provided only one of the characters is affected. Where both characters depart from the expected ratio, either gametically or zygotically, a linkage is indicated where none exists.

With departures from the expected of any kind  $P$  indicates a linkage, but in cases where the double recessive class is not recovered,  $P$  indicates but a slight departure from no linkage while both  $Q$  and  $T$  indicate perfect linkage.

Table III makes similar comparisons where there is linkage with 25 per cent crossingover. It appears that  $Q$  gives the correct result in all cases where the disturbance is zygotic except in mistaken classification and failure to recover the double recessive class. In the latter instance  $P$  is only slightly affected.  $P$  also has a slight advantage in the case where recessives are misclassified as dominant and the linkage is between dominants.

When the disturbance is gametic there is very little difference between  $Q$  and  $T$  though  $Q$  has a slight advantage in five of the six examples.

TABLE I.—Measures of linkage in a back-cross

(1) No linkage, i. e.,  $p = .5$ 

	Zygotic classes.					Observed value of $p$ .	
	AB.	Ab.	aB.	ab.	Total.	$\frac{Ab+aB}{n}$	$Q$ .
Perfect population.....	1	1	1	1	4	0.5000	0.5000
25 per cent $a$ gametes not effective.....	4	4	3	3	14	.5000	.5000
25 per cent $a$ and $b$ gametes not effective.....	16	12	12	9	49	.4898	.5000
25 per cent $a$ and $B$ gametes not effective.....	12	16	9	12	49	.5102	.5000
25 per cent $a$ zygotes wrongly classified as $A$ .....	5	5	3	3	16	.5000	.5000

(2) Dominants linked, 25 per cent crossover, i. e.,  $p = .25$ 

Perfect population.....	3	1	1	3	8	0.2500	0.2500
25 per cent $a$ gametes not effective.....	12	4	3	9	28	.2500	.2500
25 per cent $a$ and $b$ gametes not effective.....	16	4	4	9	33	.2424	.2500
25 per cent $a$ and $B$ gametes not effective.....	36	9	16	36	97	.2577	.2500
25 per cent $a$ zygotes wrongly classified as $A$ .....	13	7	3	9	32	.3125	.2976

TABLE II.—Comparison of methods of measuring linkage in  $F_2$ No linkage, i. e.,  $p = .5$ 

	Zygotic classes.					Observed value of $p$ .		
	AB.	Ab.	aB.	ab.	Total.	$Q$ .	$P$ .	$T$ .
Perfect population.....	9	3	3	1	16	0.5000	0.5000	0.5000
25 per cent $a$ gametes not effective.....	120	40	27	9	196	.5000	.5624	.5000
25 per cent $a$ and $b$ gametes not effective.....	1,600	360	360	81	2,401	.5000	.6336	.5260
25 per cent $a$ and $B$ gametes not effective.....	1,320	640	297	144	2,401	.5000	.4685	.4798
25 per cent $a$ zygotes die.....	12	4	3	1	20	.5000	.5477	.5000
25 per cent $a$ and $b$ zygotes die.....	144	36	36	9	225	.5000	.6000	.5133
25 per cent $a$ and $B$ zygotes die.....	108	48	27	12	195	.5000	.4804	.4886
25 per cent $a$ zygotes called $A$ .....	39	13	9	3	64	.5000	.5590	.5000
Double recessive class not recovered.....	9	3	3	0	15	.0000	.4472	.0000

TABLE III.—Comparison of methods for measuring linkage in  $F_2$ , 25 per cent crossing-over

	Nature of linkage.	Zygotic classes.					Value of $p$ .		
		AB.	Ab.	aB.	ab.	Total.	Q.	P.	T.
Perfect population...	D and R <sup>a</sup> ...	33	15	15	1	64	0.2500	0.2500	0.2500
	D and D...	41	7	7	9	64	0.2500	0.2500	0.2500
25 per cent $a$ gametes not effective.	D and R...	424	216	135	9	784	.2377	.5997	.2336
	D and D...	552	88	63	81	784	.2432	.2159	.2417
25 percent $a$ and $b$ gametes not effective.	D and R...	5,440	1,944	1,944	81	9,409	.2263	.4166	.2349
	D and D...	7,488	792	792	729	9,801	.2359	.1773	.2188
25 per cent $a$ and $B$ gametes not effective.	D and R...	4,824	3,456	1,377	144	9,801	.2496	.1174	.2288
	D and D...	5,976	1,408	729	1,296	9,409	.2498	.2612	.2605
25 per cent $a$ zygotes die.	D and R...	132	60	45	3	240	.2500	.3536	.2500
	D and D...	164	28	21	27	240	.2500	.2308	.2500
25 per cent $a$ and $b$ zygotes die.	D and R...	528	180	180	9	897	.2500	.4442	.2619
	D and D...	656	84	84	81	905	.2500	.2071	.2416
25 per cent $a$ and $B$ zygotes die.	D and R...	396	240	135	12	783	.2500	.2053	.2415
	D and D...	492	112	63	108	775	.2500	.2595	.2582
25 per cent $a$ called A.	D and R...	147	61	45	3	256	.2600	.4146	.2644
	D and D...	171	37	21	27	256	.2742	.2605	.2808
Double recessive not recovered.	D and R...	33	15	15	0	63	.0000	.2182	.0000

## SUMMARY

Some measure of linkage is necessary in all studies of the association between characters and chromosomes.

Back-crossing the  $F_1$  on double recessives affords the most direct measure but with many plants this method can not be used. There is also a slight though usually unimportant error in the use of this method when the Mendelian ratios depart from the expected.

Three methods have been proposed for measuring linkage in the  $F_2$ :

(1) Yule's coefficient of association,  $Q$ .

(2) Emerson's method,  $P$ .

(3) Haldane's method,  $T$ .

All of these give the correct result with a perfect population, but are variously affected by differential death rates of the gametes or zygotes, selective fertilization and mistakes in classification, which cause departures from expected Mendelian ratios.

Departures from expected Mendelian ratios are grouped into three classes and the effects of these departures on the accuracy of the three methods of measuring linkage in  $F_2$  are investigated empirically.

No one method is found to be most accurate for all classes of departures.

Yule's coefficient of association,  $Q$ , most nearly meets the requirements of a general method and a formula is given for evaluating the degree of linkage from observed values of  $Q$ .

<sup>a</sup> D and R=dominant and recessive linked, D and D=dominants linked.

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## THE OSMOTIC CONCENTRATION, SPECIFIC ELECTRICAL CONDUCTIVITY, AND CHLORID CONTENT OF THE TISSUE FLUIDS OF THE INDICATOR PLANTS OF TOOELE VALLEY, UTAH<sup>1</sup>

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### INTRODUCTION

During 1911-1913 Kearney, Briggs, Shantz, McLane, and Piemeisel (24)<sup>2</sup> conducted a detailed investigation of the relationship between the plant associations and the soil conditions of Tooele Valley, Utah. The purpose of this work was to ascertain to what extent natural vegetation may serve as an index of soil conditions, and in consequence as an indicator of the suitability for agricultural development of the soils of a dry and in part highly saline region. The work was a natural outcome of the interest in indicator vegetation aroused by a paper on the plains region by Shantz (27).

With the exception of early papers by Hilgard and his associates, these were pioneer investigations on the utilization of the native vegetation of the United States as a means of estimating the adaptability of the soil for agricultural purposes. The problem of indicator vegetation has since been treated more extensively by Clements (2).

The investigations by our predecessors (24) included extensive determinations on the physical and chemical characteristics of the soil. They investigated moisture content, moisture equivalent, wilting coefficient, salt content, and electrical conductivity of the soil solution as measured by the Wheatstone bridge, in typical areas of the more important plant associations. Their studies show that it is possible from an inspection of the vegetation to draw fairly definite conclusions concerning the physical and chemical properties of the soil. They correlated these two kinds of information with the results of agricultural experience in this region, which has long been the locus of limited but intensive and intelligent crop production, both with and without irrigation.

At the time these studies were made, methods for the precise measurement of the physicochemical properties of plant saps in the field were not available. It was not, therefore, possible for these authors to

<sup>1</sup> Received for publication Feb. 16, 1924.

<sup>2</sup> Reference is made by number (italic) to "Literature cited," p. 922-924.

consider the physical and chemical properties of the plant tissue fluids in the same way that they did the physical and chemical properties of the soil.

Since their studies were completed, much progress has been made in the investigation of the properties of the plant tissue fluids in relation to environmental factors. The pioneer plasmolytic studies of Drabble and Drabble (4) in the little diversified English habitats and of Fitting (5) in the extreme conditions of the North African deserts have been followed by more exact measurements by the cryoscopic method carried out in the Arizona deserts (12), in coastal deserts (15), in the mangrove swamps (16), in the mesophytic habitats of the eastern United States (11), where electrical conductivity of the sap has also been investigated (20), and in the Montane Rain Forest of Jamaica (17). The results of these studies, which have been reinforced in their significance by parallel investigations on the tissue fluids of epiphytic plants (18) and on those of parasite and host in the case of both rain-forest (13) and desert Lorantheaceae (19) point clearly to a close relationship between the physicochemical properties of the leaf tissue fluids of the plant and its geographic distribution. The significance of this relationship has been emphasized elsewhere (14).<sup>3</sup>

#### METHODS

The methods employed are those used in a series of investigations published by the writers during the past several years.

Tissues were collected in heavy-walled glass tubes and were thoroughly frozen in an ice-salt mixture for at least 10 hours (6) in order to render the tissues permeable as has been shown to be necessary by Dixon and Atkins (3) and by Gortner, Lawrence, and Harris (7). Sap was then extracted by pressure in a heavily tinned steel press bowl and was centrifuged before the determination of the constants. Freezing point lowering,  $\Delta$ , was determined by means of a thermometer graduated to hundredths of degrees.<sup>4</sup>

Correction for the concentration caused by undercooling was made by the usual formula. The results are expressed in degrees of freezing point lowering,  $\Delta$ , and in atmospheres pressure,  $P$ , as calculated from a well known formula (9), and tabled up to  $\Delta = 5.99^\circ$  (10).

Specific electrical conductivity was determined at  $30^\circ$  in a Freas conductivity cell standardized against N/10 KCl taken as having a specific electrical conductivity at  $30^\circ$  of 0.01412 mho.

<sup>3</sup> The possible bearing of such investigations on agriculture has been definitely in mind from the earliest stage of the work. A favorable opportunity for initiating studies of the properties of the tissue fluids in relation to agricultural problems presented itself in 1919 when Dr. T. H. Kearney, Physiologist, in charge of Alkali and Drought Resistant Plant Investigations, and Mr. G. N. Collins, Botanist, in charge of Biophysical Investigations, of the Bureau of Plant Industry, United States Department of Agriculture, suggested that it would be desirable to ascertain to what extent the physicochemical properties of the tissue fluid of the plants differ from association to association in response to the differences in soil conditions.

As a result of this suggestion, work was undertaken in Tooele Valley in May, 1920. Grantsville was selected as a base because of the ready accessibility of all of the associations. The following pages give the results of determinations carried out in this region in May, June, and July. While a certain number of supplementary observations were made in the Stansbury Mountains, in Rush Valley and in the Sevier Desert to the south, the results presented here are mainly restricted to the plant associations treated by Kearney, Briggs, Shantz, McLane, and Piemeisel.

Since our primary object in this investigation was to reinvestigate from an entirely new side the problem considered by our predecessors in the field, we have made every effort to follow as exactly as possible the classification of vegetation adopted by them. The present paper is therefore incomplete except as it is considered in connection with the survey already cited. For this reason a detailed description of habitats and of the general features of the vegetation is superfluous.

It is a pleasure to acknowledge our great obligation to Ivar Tidestrom of the Office of Economic and Systematic Botany, Bureau of Plant Industry, United States Department of Agriculture, for his painstaking work in the identification of our herbarium materials.

<sup>4</sup> In the case of some of the more concentrated saps a thermometer of  $+1$  to  $-10$  degrees range graduated in one-fiftieth degree was of necessity used instead of the one graduated in one-hundredth degree units.



At the time the field work was done the writers were not acquainted with Mason's (26) attempt to correct conductivities for the viscosity of the solution by determining the increase in conductivity due to the addition of a given amount of KCl to the tissue fluids. The conductivities as given here are, therefore, the raw values, wholly uncorrected for the viscosity of the sap. It is perhaps obvious that it would have been impossible in dealing with plants with such a high chlorid content as those of the Great Salt Lake region to utilize KCl in determining the influence of viscosity on the conductivity, but it may be unfortunate that some other salt could not have been employed for this purpose.

Some measure of the relative importance of salts and organic solutes in determining the osmotic concentration is highly desirable. We have used for the purpose the ratio of specific electrical conductivity,  $K$ , to freezing point lowering,  $\Delta$ .

Chlorid content was determined on samples of sap pipetted in the field and preserved in sealed tubes for subsequent analysis. The analytical method employed is described elsewhere (25). The results are expressed in terms of grams of chlorids Cl per liter of tissue fluid.

#### PRESENTATION OF DATA

In the presentation and analysis of the measurements we have followed as closely as possible the classification of plant associations recognized by Kearney and his coworkers (24). Since growth forms have been shown to be differentiated in their sap properties by our earlier studies on the vegetation of the Arizona deserts (12) and by more recent investigations in humid (17) and mesophytic (20) habitats, it is essential that the species of each habitat be classified according to growth form. Since the herbaceous species are chiefly evanescent, it would be improper to include them with the ligneous species in obtaining a general average for the association unless it be shown that the concentrations of the tissue fluids of the ligneous species remain the same throughout the season.

Because of the concentration of the soil solution due to drying, and due also, perhaps, to some extent to the accumulation near the surface of salts brought up from lower levels, it seems altogether unlikely that concentration of the tissue fluids of the more permanent species of the halophytic vegetation will remain the same throughout the season. Such a result is quite contrary to the findings of Cavara (1) who investigated the changes in a number of halophytes with the march of the season. It is therefore necessary in the analysis of the data to arrange the determinations chronologically. It is impossible in one year's work to obtain satisfactory data sufficient in quantity to show the seasonal change in each of the habitats for even the more important species. The results of the present series must serve as merely a rough indication of seasonal change.

An earlier discussion of the plant associations of this region (24) emphasized the sharpness of demarcation which characterizes their distribution. In our discussion of the constants it may appear that there have been considerable difficulties in deciding to which association some of the samples should be assigned. This is largely due to the fact that from many sides the narrow transition zones, or those which afford a complex of physical conditions, are biologically the most interesting. Such conditions are found, for example, in the washes through the sagebrush

and the shadscale associations, in the localities where springs occur along the margins of the salt flats, and in the small islands of *Kochia* in the sagebrush association. Such localities give the opportunity of investigating in close association pairs of species which are normally different in their distribution. It has been necessary to explain certain of these cases, and this has laid wholly undue emphasis on the indefinite nature of the transitions, which in general are quite as sharp and definite as our predecessors have indicated.

Another factor tending to produce an apparent difference in the results of the two studies of the region lies in the fact that in seeking for materials for physiological determinations the writers availed themselves of tissues of a single plant, or of but a few individuals of the rarer species. These would be practically left out of account by the descriptive ecologist in mapping the vegetation. Thus from the descriptions and species lists of the writers it might appear that Kearney and his associates over-emphasized the small number of the dominant species. This is not at all the case.

#### STANSBURY MOUNTAINS AT HIGHER ALTITUDES

The highest zone of Tooele Valley which is of agricultural (distinguished from grazing and water conservation) interest is that formed by the great alluvial cones or fans (outwash slopes). These are, or have in all probability been in times past, largely covered by the sagebrush association.

While Kearney and his party carried their observations no higher than the sagebrush association of these broad slopes which border the steeper foothills, it has seemed desirable in a consideration of the tissue fluids of the plants of the region, to include a few determinations made at higher altitudes in the Stansbury Mountains. These may serve as a basis of comparison with those taken in the desert valley.

The species mentioned give some idea of the associations to which the plants belong. A detailed discussion of the habitats is omitted, since we understand that Dr. C. F. Korstian, who has given much attention to the Wasatch Mountains, will treat the problem of the tissue fluids of the plant associations of these mountains in considerable detail.

The constants for the species of this association appear in Table I.

TABLE I.—Physicochemical constants for species of the Stansbury Mountains at higher altitudes

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
TREES						
	1920.					
<i>Pinus flexilis</i> James.....	July 23	1.44	17.3	0.0086	0.0059	0.4
<i>Pseudotsuga mucronata</i> (Raf.) Sudw.	June 21	1.85	22.3	0.0087	0.0047	0.7
	July 23	1.50	18.0	0.0081	0.0054	.....
Average.....		1.68	20.2	0.0084	0.0050	0.7
<i>Populus aurea</i> Tidest.....	June 21	1.54	18.5	0.0137	0.0089	0.3
	July 23	1.81	21.8	0.0099	0.0055	0.2
Average.....		1.68	20.2	0.0118	0.0072	0.3

TABLE I.—Physicochemical constants for species of the Stansbury Mountains at higher altitudes—Continued

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
TREES—continued						
	1920.					
<i>Acer glabrum</i> Torr. ....	July 23	1.00	12.0	0.0125	0.0113	0.8
	...do....	0.90	10.8	0.0133	0.0148	0.6
Average.....		0.95	11.4	0.0129	0.0130	0.7
<i>Prunus melanocarpa</i> (A. Nels.) Rydb.....	June 21	1.54	18.5	0.0122	0.0080	0.3
<i>Salix scouleriana</i> Barr.....	July 23	1.18	14.2	.....	.....	.....
SHRUBS AND HALF SHRUBS						
<i>Artemisia tridentata</i> Nutt. ....	July 23	1.62	19.5	0.0164	0.0101	2.2
<i>Cercocarpus ledifolius</i> Nutt. ....	June 21	1.44	17.3	0.0067	0.0047	0.8
	July 23	1.89	22.7	0.0068	0.0036	0.2
Average.....		1.67	20.0	0.0068	0.0041	0.5
<i>Opulaster malvaceus</i> (Greene) Kuntze.....	June 21	1.19	14.3	0.0144	0.0121	0.3
	July 23	1.56	18.8	0.0122	0.0078	0.4
Average.....		1.38	16.6	0.0133	0.0100	0.4
<i>Pachystima myrsinites</i> (Pursh) Raf..	June 21	1.26	15.2	0.0093	0.0074	1.3
<i>Sambucus microbotrys</i> Rydb.....	...do....	1.06	12.8	0.0247	0.0232	0.2
	July 23	1.09	13.2	0.0184	0.0168	0.8
Average.....		1.08	13.0	0.0215	0.0200	0.5
<i>Sorbus scopulina</i> Greene.....	June 21	1.55	18.6	0.0136	0.0088	0.2
HERBS						
<i>Aquilegia thalictrifolia</i> Rydb.....	July 23	1.53	18.3	.....	.....	.....
<i>Aquilegia truncata</i> Fisch. & Mey....	...do....	1.28	15.5	0.0156	0.0122	.....
<i>Arnica longifolia</i> D. C. Eat.....	...do....	0.87	10.5	0.0213	0.0244	1.7
<i>Balsamorhiza sagittata</i> (Pursh) Nutt.	June 21	1.27	15.3	0.0221	0.0174	1.3
<i>Castilleja linariaefolia</i> Benth.....	July 23	1.70	20.4	0.0189	0.0111	1.9
<i>Claytonia lanceolata</i> Pursh.....	June 21	0.59	7.1	0.0147	0.0251	0.2
<i>Cogswellia grayii</i> C. & R.....	...do....	1.47	17.6	0.0203	0.0138	1.6
<i>Cynomarathrum Nuttallii</i> A. Gray....	...do....	1.14	13.7	0.0184	0.0161	3.0
	July 23	1.02	12.3	0.0210	0.0206	2.3
Average.....		1.08	13.0	0.0197	0.0184	2.7
<i>Eriogonum subalpinum</i> Greene....	June 21	1.21	14.6	0.0141	0.0116	1.7
	July 23	1.68	20.2	0.0178	0.0106	2.3
Average.....		1.45	17.4	0.0160	0.0111	2.0
<i>Erythronium parviflorum</i> (S. Wats.) Goodd.....	June 21	0.99	11.9	0.0093	0.0095	0.5
	...do....	0.96	11.5	0.0099	0.0104	0.5
Average.....		0.98	11.7	0.0096	0.0099	0.5
<i>Frasera</i> sp. ....	June 21	0.76	9.1	0.0071	0.0094	.....
<i>Frasera spectiosa</i> Dougl.....	July 23	1.00	12.0	0.0100	0.0100	1.4

TABLE I.—Physicochemical constants for species of the Stansbury Mountains at higher altitudes—Continued

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio conductivity to depression, K/ $\Delta$	Chlorids per liter, Cl.
HERBS—continued						
<i>Geranium richardsonii</i> Fisch. & Trautv.....	1920. July 23	0.78	9.4	0.0127	0.0163	1.1
<i>Heuchera flavescens</i> Rydb.....	June 21	0.87	10.5	0.0119	0.0137	0.7
<i>Hydrophyllum watsoni</i> (A. Gray) Rydb.....	do.....	0.68	8.2	0.0193	0.0283	0.3
<i>Lathyrus ulahensis</i> Jones.....	do.....	0.95	11.5	0.0174	0.0183	1.6
<i>Leptotaenia multifida</i> Nutt.....	do.....	1.31	15.8	0.0175	0.0133	1.1
	do.....	1.35	16.2	0.0238	0.0176	1.1
Average.....		1.33	16.0	0.0206	0.0155	1.1
<i>Ligusticum filicinum</i> S. Wats.....	July 23	1.28	15.5	0.0236	0.0184	.....
<i>Mertensia foliosa</i> A. Nels.....	June 21	0.95	11.4	0.0225	0.0238	3.3
<i>Mimulus lewisii</i> Pursh.....	July 23	0.75	9.0	0.0134	0.0179	1.1
<i>Mitella stauropetala</i> Piper.....	June 21	0.93	11.2	0.0169	0.0181	0.3
<i>Penstemon procerus</i> Dougl.....	do.....	1.10	13.3	0.0128	0.0117	0.1
<i>Rudbeckia occidentalis</i> Nutt.....	July 23	0.90	10.9	0.0152	0.0169	1.4
<i>Senecio eremophilus</i> Richards.....	do.....	0.74	8.9	0.0193	0.0263	2.3
<i>Solidago pumila</i> Nutt.....	do.....	1.46	17.6	0.0221	0.0151	2.6
<i>Stellaria jamesiana</i> Torr.....	June 21	1.13	13.6	0.0230	0.0204	1.7
<i>Thalictrum</i> sp.....	July 23	1.67	20.1	0.0209	0.0125	1.8
<i>Veratrum speciosum</i> Rydb.....	do.....	1.05	12.6	0.0172	0.0164	0.1
<i>Wyethia amplexicaulis</i> Nutt.....	July 28	1.19	14.3	0.0193	0.0163	2.8

Some of the differences between two or more determinations on the same species may be due to differences in the age of the leaves. In some instances the snow had but recently disappeared from the ground, and the leaves were much younger in the earlier than in the later of the two collections. This is true of the determinations on *Populus* and *Opulaster*. Some of the determinations are based on plants from stream banks, while others were obtained from the drier slopes. For present purposes it is not worth while to go into the details of local distribution of the montane vegetation, which is included merely as a basis of rough comparison with the desert habitats.

Discussion of the results for the individual habitats will be reserved until they can be compared with others. This will be given in part in connection with the data of the following plant associations, and in part in a general summary.

#### SAGEBRUSH ASSOCIATION

In Tooele Valley the sagebrush association, which is one of the most important types of vegetation in the Great Basin region, occurs chiefly on the bench lands or upper outwash slopes which skirt the mountains. It is the characteristic plant covering of the alluvial fans which represent the enormous accumulations of detritus swept out from the mouths of the canyons. From the agricultural standpoint, the sagebrush association is of great interest, since it is upon this land that the dry-farm cultivation of the small grains is chiefly conducted.

The lower limits of the sagebrush association are rather sharply defined, and are marked by the upper limits of the shadscale association,

of the *Kochia* association, of the greasewood-shadscale association, or even, in places, of the salt flats.

The upper limit of the association is far less definite, since sagebrush extends to considerable elevations and is an important element in the vegetation of the mountains. For the purpose of the present paper, we have taken the upper limit of the sagebrush association to be equivalent to the elevation at which cedars become an important element in the vegetation.

In order to obtain a basis of comparison for the vegetation of the drier or more saline habitats, we have found it desirable to make a few determinations on species growing in the mouths of the canyons. We have, therefore, divided the sagebrush association into two parts for convenience of treatment—the sagebrush association of the alluvial fans and the sagebrush association of the foothills canyons.

Since the washes which are not occupied by permanent streams diversify the topography and soil conditions and afford moisture in greater quantities and for longer periods than do the relatively uniform and undiversified slopes of the alluvial fans, it has seemed desirable to divide the sagebrush association of the alluvial fans into two sections, one of which treats of the typical sagebrush association while the other deals with the vegetation of the generally dry watercourse or washes through the sagebrush association.

On *a priori* grounds, one might expect that the osmotic concentration of the plants of the typical sagebrush association would be somewhat greater than that of those bordering the washes or of the stream beds, which are generally dry during the summer season, at the mouths of the canyons.

Our predecessors (24) conclude that in Tooele Valley the soil conditions for a good stand and development of sagebrush are, (a) a rather coarse-textured soil readily permeable to water, with a small run-off and good underdrainage, (b) a depth of soil of at least 3 feet in which water can be stored and into which roots can penetrate, and (c) at least 3 feet of soil in which the salt content is very low.

These conditions are best met on the upper outwash slopes and on the sand hills. Poorly developed plants of sagebrush may occur in other localities, as in the *Kochia* association, and even in the greasewood-shadscale association, but in such localities the development is highly abnormal, and the tissue fluids may be characterized by very high concentrations.

The determinations on the herbaceous species of the sagebrush association were of necessity made chiefly during the early part of the season, since they are practically all of the ephemeral type. Most of these species develop with great rapidity in the early part of the season when the soil moisture has not yet been exhausted from the upper foot or two of soil. By early summer they have fruited and died down to the ground. They are, therefore, "drought evading" rather than "drought resisting" (23), corresponding in this regard to the winter annuals of the Arizona deserts.

#### THE SAGEBRUSH ASSOCIATION OF THE FOOTHILLS CANYONS

This division has been included largely as a means of securing a basis of comparison with the drier or more saline areas of the broad valley. It is perhaps misnamed, since the plants considered are practically entirely taken from the banks of the watercourses where sagebrush is not the dominant element in the vegetation. Sagebrush is, however, the chief ligneous plant on the slopes of the canyon walls.

This division of the sagebrush association has been given first place in the series, since it is the highest in altitude of the three divisions and most closely related in respect to both physical conditions and vegetation to the preceding associations, grouped under the general term of "Stansbury Mountains at higher altitudes."

The following determinations (Table II) were based on collections made along the lower course of Box Elder Creek.<sup>5</sup>

TABLE II.—Physicochemical constants for species of the sagebrush association of the foothills canyons

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
TREES AND SHRUBS						
	1920.					
<i>Acer interius</i> Britton .....	July 16	1.20	14.4	0.0147	0.0123	0.8
<i>Ribes inebrians</i> Lindl. ....	do.	1.57	18.8	0.0156	0.0099	1.8
<i>Sambucus coerulea</i> Raf. ....	do.	1.39	16.7	0.0163	0.017	0.1
<i>Symphoricarpos oreophilus</i> A. Gray.	July 28	2.34	28.1	0.0148	0.0063	0.8
PERENNIAL HERBS						
<i>Agastache urticifolia</i> (Benth.) Rydb.	July 16	0.95	11.5	0.0219	0.0230	2.1
<i>Artemisia dracunculoides</i> Pursh. ....	do.	1.05	12.6	0.0208	0.0199	3.8
<i>Artemisia ludoviciana</i> Nutt. ....	do.	1.04	12.6	0.0205	0.0197	3.1
<i>Castilleja linariaefolia</i> Benth. ....	do.	1.86	22.4	0.0258	0.0138	.....
<i>Cirsium lanceolatum</i> (L.) Hill. ....	do.	1.08	13.0	0.0259	0.0240	4.1
<i>Elymus condensatus</i> Presl. ....	do.	2.08	25.0	0.0357	0.0172	5.1
<i>Eriogonum elatum</i> Dougl. ....	do.	1.23	14.8	0.0194	0.0158	6.9
<i>Eriogonum subalpinum</i> Greene. ....	do.	1.26	15.1	0.0153	0.0122	2.8
	July 28	2.07	24.8	0.0176	0.0085	3.8
Average. ....		1.67	20.0	0.0164	0.0103	3.3
<i>Geranium viscosissimum</i> Fisch. & Mey. ....	July 16	1.04	12.5	0.0170	0.0164	3.0
<i>Hedysarum pabulare</i> A. Nels. ....	July 17	1.21	14.6	0.0186	0.0153	3.7
<i>Lappula floribunda</i> (Lehm.) Greene	July 16	1.11	13.3	0.0254	0.0230	3.0
<i>Lithospermum torreyi</i> Nutt. ....	do.	1.21	14.5	0.0235	0.0194	4.1
<i>Marrubium vulgare</i> L. ....	do.	1.11	13.3	0.0256	0.0232	5.2
<i>Pachylophus marginatus</i> (Nutt.) Rydb. ....	do.	0.83	10.0	0.0192	0.0231	2.7
<i>Rumex crispus</i> L. ....	do.	0.89	10.7	0.0233	0.0263	2.7
<i>Scrophularia occidentalis</i> (Rydb.) Bickn. ....	do.	1.01	12.1	0.0174	0.0173	.....
<i>Solidago pumila</i> . ....	July 28	1.82	21.9	0.0274	0.0150	4.9
<i>Sphaeralcea rivularis</i> Torr. ....	July 16	1.40	16.8	0.0283	0.0202	.....
<i>Vagnera stellata</i> (L.) Morong. ....	do.	1.23	14.8	0.0203	0.0166	3.3
BIENNIAL HERBS						
<i>Mentzelia acuminata</i> (Rydb.) .....	July 16	1.05	12.7	0.0236	0.0224	.....
<i>Oenothera</i> sp. ....	do.	0.81	9.7	0.0188	0.0232	2.7
<i>Oenothera hookeri</i> Torr. & G. ....	do.	0.72	8.7	0.0187	0.0260	.....
<i>Oenothera strigosa</i> (Rydb.) Mack. & Bush. ....	do.	0.71	8.5	0.0173	0.0244	3.2
ANNUAL HERBS						
<i>Lactuca scariola</i> L. ....	July 16	0.86	10.3	0.0207	0.0241	2.6
<i>Urtica breweri</i> S. Wats. ....	do.	1.02	12.2	0.0221	0.0218	3.4
<i>Urtica gracilis</i> Ait. ....	do.	1.32	15.8	0.0210	0.0159	2.2

<sup>5</sup> Unfortunately the sagebrush itself was omitted from the two collections made in this habitat.

## WASHES THROUGH THE SAGEBRUSH ASSOCIATION

The constants for the species of this association are presented in Table III.

TABLE. III.—Physicochemical constants for species of the washes through the sagebrush association.

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
TREES, SHRUBS, AND HALF SHRUBS						
<i>Juniperus utahensis</i> (Engelm.)	1920.					
Lemmon.....	July 14	2.10	25.3	.....	.....	0.2
<i>Artemisia tridentata</i> Nutt.....	July 12	2.62	31.4	0.0243	0.0093	4.4
<i>Atriplex canescens</i> (Pursh) Nutt.....	do.....	3.25	39.0	0.0512	0.0157	.....
	July 14	2.49	29.9	0.0397	0.0159	7.4
Average.....		2.87	34.5	0.0454	0.0158	7.4
<i>Chrysothamnus</i> sp.....	July 12	1.51	18.1	0.0230	0.0153	2.9
Do.....	do.....	1.71	20.5	0.0223	0.0131	3.1
<i>Chrysothamnus lanceolatus</i> Nutt.....	do.....	1.61	19.4	0.0261	0.0162	4.7
<i>Ribes aureum</i> Pursh.....	do.....	1.61	19.4	0.0169	0.0105	2.5
<i>Symphoricarpos oreophilus</i> A. Gray.....	do.....	2.07	24.9	0.0134	0.0065	0.8
<i>Kochia vestita</i> S. Wats.....	do.....	2.53	30.4	0.0449	0.0179	3.8
HERBS						
<i>Argemone hispida</i> A. Gray.....	do.....	1.09	13.1	0.0170	0.0156	0.5
<i>Artémisia dracunculoides</i> Pursh.....	do.....	1.27	15.3	0.0168	0.0132	2.2
<i>Coleosanthus linifolius</i> (D. C. Eat.)						
Kuntze.....	do.....	1.64	19.7	0.0283	0.0173	4.7
<i>Marrubium vulgare</i> L.....	do.....	1.72	20.7	0.0277	0.0160	4.4
<i>Mentzelia acuminata</i> (Rydb.).....	do.....	1.29	15.5	0.0204	0.0158	1.7
<i>Verbascum thapsus</i> L.....	do.....	1.26	14.4	0.0191	0.0159	2.5
<i>Verbena bracteosa</i> Michx.....	do.....	1.30	15.6	0.0202	0.0156	3.3

## THE SAGEBRUSH ASSOCIATION OF THE ALLUVIAL FANS

The constants for the species of this association appear in Table IV.

TABLE IV.—Physicochemical constants for species of the sagebrush association of the alluvial fans

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS						
<i>Juniperus utahensis</i> (Engelm.)	1920.					
Lemmon.....	June 7	1.74	20.9	0.0097	0.0056	0.6
<i>Artemisia tridentata</i> Nutt.....	June 5	1.38	16.6	0.0208	0.0151	.....
	June 7	1.56	18.8	0.0190	0.0122	2.1
	June 9	1.45	17.4	0.0199	0.0138	2.2
	June 15	1.69	20.3	0.0222	0.0131	2.9
	July 16	2.50	30.0	0.0210	0.0084	3.6
Average.....		1.72	20.6	0.0206	0.0125	2.7

TABLE IV.—Physicochemical constants for species of the sagebrush association of the alluvial fans—Continued

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS—contd.						
	1920.					
<i>Chrysothamnus</i> sp. ....	June 5	1.42	17.0	0.0201	0.0141	.....
Do. ....	June 15	1.35	16.3	0.0260	0.0192	3.8
Do. ....	do. ....	1.49	17.9	0.0236	0.0158	3.5
<i>Chrysothamnus latifolius</i> (D. C. Eat.) Rydb. ....	do. ....	1.41	17.0	0.0248	0.0176	3.9
<i>Atriplex canescens</i> (Pursh) Nutt. ....	June 5	1.86	22.4	0.0354	0.0190	.....
.....	June 15	1.80	21.7	0.0381	0.0213	7.7
Average. ....		1.83	22.1	0.0369	0.0201	7.7
<i>Atriplex confertifolia</i> (Torr.) S. Wats. ....	June 15	3.09	37.0	0.0652	0.0212	.....
<i>Tetradymia nuttallii</i> T. & G. ....	June 5	1.55	18.6	0.0342	0.0221	.....
.....	June 15	1.60	19.2	0.0308	0.0193	0.7
Average. ....		1.58	18.9	0.0325	0.0207	0.7
<i>Ribes inebrians</i> Lindl. ....	June 7	1.60	19.3	0.0214	0.0134	2.8
<i>Rhus trilobata</i> Nutt. ....	June 15	1.58	19.0	0.0138	0.0087	1.8
<i>Symphoricarpos oreophilus</i> A. Gray. ....	June 9	1.86	22.4	0.0116	0.0062	0.2
PERENNIAL HERBS						
<i>Agoseris pumila</i> (Nutt.) Rydb. ....	June 9	0.94	11.4	0.0226	0.0239	4.1
.....	do. ....	0.92	11.1	0.0213	0.0232	2.5
Average. ....		0.93	11.3	0.0220	0.0236	3.3
<i>Agoseris elata</i> (Nutt.) Greene. ....	June 7	0.90	10.9	0.0228	0.0252	3.3
<i>Agropyron spicatum</i> (Pursh) S. & S. ....	June 15	1.58	19.0	0.0333	0.0211	2.9
<i>Allionia linearis</i> Pursh. ....	do. ....	0.91	11.0	0.0228	0.0249	2.8
<i>Astragalus</i> sp. ....	do. ....	1.21	14.6	0.0175	0.0145	2.5
<i>Astragalus cibarius</i> Sheldon. ....	June 7	1.37	16.5	0.0166	0.0121	1.0
.....	June 9	1.36	16.4	0.0193	0.0141	1.1
Average. ....		1.37	16.5	0.0179	0.0131	1.1
<i>Astragalus utahensis</i> T. & G. ....	June 7	1.12	13.4	0.0183	0.0165	1.8
<i>Balsamorhiza hirsuta</i> Nutt. ....	do. ....	1.09	13.1	0.0241	0.0221	1.6
<i>Balsamorhiza sagittata</i> (Pursh) Nutt. ....	June 9	1.22	14.7	0.0258	0.0211	2.0
<i>Castilleja hispida</i> Benth. ....	June 7	1.49	17.9	0.0330	0.0221	1.4
.....	June 9	1.55	18.6	0.0331	0.0213	1.4
Average. ....		1.52	18.3	0.0330	0.0217	1.4
<i>Chaenactis stevioides</i> H. & A. ....	June 7	1.11	13.4	0.0232	0.0209	.....
.....	June 9	1.07	12.9	0.0235	0.0218	4.4
Average. ....		1.09	13.2	0.0234	0.0214	4.4
<i>Cirsium neomexicanum</i> A. Gray. ....	June 7	1.15	13.9	0.0252	0.0219	5.4
<i>Crepis gracilis</i> (D. C. Eat.) Rydb. ....	do. ....	1.12	13.5	0.0260	0.0231	4.5
<i>Erigeron pumilus</i> Nutt. ....	do. ....	1.32	15.8	0.0272	0.0207	4.5
<i>Eriogonum elatum</i> Dougl. ....	do. ....	1.19	14.4	0.0191	0.0180	2.5
.....	June 9	1.18	14.2	0.0179	0.0151	2.3
Average. ....		1.19	14.3	0.0185	0.0156	2.4



TABLE IV.—Physicochemical constants for species of the sagebrush association of the alluvial fans—Continued

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
PERENNIAL HERBS—continued						
	1920.					
<i>Eriogonum ovalifolium</i> Nutt. ....	June 7	1.32	15.8	0.0164	0.0125	2.4
<i>Hedysarum utahense</i> Rydb. ....	do. ....	1.22	14.7	0.0152	0.0125	1.8
	June 9	1.21	14.5	0.0185	0.0153	2.2
Average. ....		1.22	14.6	0.0169	0.0139	2.0
<i>Lappula floribunda</i> (Lehm.) Greene	June 7	0.97	11.7	0.0214	0.0220	1.6
	June 9	0.93	11.2	0.0200	0.0215	1.5
Average. ....		0.95	11.5	0.0207	0.0217	1.6
<i>Lithospermum torreyi</i> Nutt. ....	June 7	1.10	13.3	0.0217	0.0197	3.8
<i>Oreocarya</i> sp. ....	do. ....	1.03	12.4	0.0206	0.0200	1.8
<i>Phlox longifolia</i> Nutt. ....	June 9	1.24	14.9	0.0143	0.0116	0.8
<i>Phlox stansburyi</i> (Torr.) Heller. ....	June 7	1.13	13.6	0.0131	0.0116	1.3
<i>Senecio hydrophilus</i> Nutt. ....	do. ....	0.86	10.3	0.0254	0.0296	2.6
<i>Senecio multilobatus</i> T. & G. ....	June 6	0.94	11.3	0.0190	0.0201	.....
	June 9	0.97	11.7	0.0152	0.0157	.....
Average. ....		0.96	11.5	0.0171	0.0179	.....
<i>Stenotus acaulis</i> Nutt. ....	June 7	0.97	11.7	0.0177	0.0182	2.0
	do. ....	1.16	13.9	0.0201	0.0174	2.7
Average. ....		1.07	12.8	0.0189	0.0178	2.4
<i>Vicia americana</i> Muhl. ....	June 9	1.28	15.4	0.0182	0.0142	1.0
<i>Wyethia amplexicaulis</i> Nutt. ....	do. ....	1.23	14.7	0.0239	0.0195	3.8
<i>Zygadenus paniculatus</i> S. Wats. ....	do. ....	1.05	12.7	0.0106	0.0101	0.1
ANNUAL HERBS						
<i>Amsinckia tessellata</i> A. Gray. ....	June 5	0.85	10.2	0.0209	0.0246	1.9
<i>Argemone hispida</i> A. Gray. ....	June 15	1.00	12.0	0.0180	0.0181	0.8
<i>Argemone rotundata</i> Rydb. ....	June 5	0.97	11.7	0.0150	0.0155	.....
<i>Camelina microcarpa</i> Andr. ....	June 7	0.95	11.5	0.0181	0.0191	.....
	June 9	0.97	11.7	0.0147	0.0151	3.3
Average. ....		0.96	11.6	0.0164	0.0171	3.3
<i>Erodium cicutarium</i> (L.) L'Her. ....	June 9	1.02	12.3	0.0201	0.0196	0.9
<i>Sophia filipes</i> (A. Gray) Heller. ....	do. ....	1.27	15.3	0.0187	0.0147	1.0

## SAND-HILL, MIXED ASSOCIATION

Sand dunes or sand hills clothed with vegetation constitute but an insignificant part of the area of Tooele Valley.

We have nothing to add to the general description of the association as given by our predecessors. From their rather limited studies of the physical conditions, they concluded that the soil is characterized by (a) a low moisture-holding capacity, as indicated by the low moisture equivalent, by (b) the presence of available moisture, at least during the earlier part of the season, at a depth attainable by the more deeply penetrating roots, and by (c) a very low salt content.

The location of this association, as we examined it, in the path of the winds sweeping over Stockton bar from the southwest, results in the early desiccation of all the herbaceous vegetation. Only the few deeper-rooted woody species are available later in the season. Our series of determinations is, therefore, limited.

The available constants for the species of this association are given in Table V.

TABLE V.—Physicochemical constants for species of the sand-hill mixed association

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorides per liter, Cl.
TREES AND SHRUBS						
<i>Juniperus utahensis</i> (Engelm.)	1920.					
Lemmon.....	June 3	1.85	22.3	0.0118	0.0063	1.7
	June 11	1.78	21.4	0.0127	0.0071	1.3
Average.....		1.82	21.9	0.0122	0.0067	1.5
<i>Artemisia tridentata</i> Nutt.....	June 3	1.59	19.2	0.0179	0.0112	2.7
	June 11	1.61	19.4	0.0221	0.0137	2.6
Average.....		1.60	19.3	0.0200	0.0125	2.7
<i>Grayia spinosa</i> (Hook.) Moq.....	June 3	1.72	20.7	0.0429	0.0249	4.5
ANNUAL HERBS						
<i>Abronia salsa</i> Rydb.....	June 3	0.87	10.4	0.0210	0.0243	.....
	June 11	0.88	10.6	0.0248	0.0282	1.6
Average.....		0.88	10.5	0.0229	0.0262	1.6
<i>Balsamorhiza hirsuta</i> Nutt.....	June 3	1.09	13.1	0.0203	0.0187	1.4
<i>Anogra pallida</i> (Dougl.) Britt. ....	do....	0.85	10.3	0.0145	0.0170	2.0
	June 11	0.79	9.5	0.0163	0.0208	1.6
Average.....		0.82	9.9	0.0154	0.0189	1.8
<i>Castilleja hispida</i> Benth.....	June 3	1.52	18.3	0.0316	0.0207	2.7
	June 11	1.80	21.6	0.0342	0.0190	2.8
Average.....		1.66	20.0	0.0329	0.0199	2.8
<i>Chaenactis stevioides</i> H. & A.....	June 3	0.94	11.3	0.0223	0.0237	.....
<i>Delphinium</i> sp.....	do....	1.30	15.6	0.0271	0.0208	1.6
<i>Eriogonum ovalifolium</i> Nutt.....	do....	1.21	14.6	0.0163	0.0134	2.9
	June 11	1.23	14.8	0.0179	0.0146	.....
Average.....		1.22	14.7	0.0171	0.0140	2.9
<i>Oreocarya</i> sp.....	June 3	0.89	10.7	0.0191	0.0215	1.7
<i>Zygadenus paniculatus</i> S. Wats.....	June 11	1.11	13.4	0.0115	0.0104	0.2

#### KOCHIA ASSOCIATION

The Kochia association, composed almost exclusively of the half-shrub, *Kochia vestita*, occupies a narrow belt extending nearly across the valley between the lower limits of the sagebrush association and the upper limits of the shadscale association.

Other ligneous species are in general limited in occurrence to the edges of the washes, although very dwarfed sagebrush may occur sporadically. A few ephemeral annuals which complete their growth relatively early in the season also occur.

Because of the small number of species and the remarkably small variability of the type species, the *Kochia* association is the most uniform in appearance of any type of vegetation occurring in the valley.

TABLE VI.—Physicochemical constants for species of the *Kochia* association

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS						
	1920.					
<i>Kochia vestita</i> S. Wats.....	June 9	1.96	23.6	0.0444	0.0226	4.4
	June 15	2.40	28.8	0.0527	0.0220	5.4
..do....	..do....	2.58	31.0	0.0589	0.0228	7.5
	June 17	2.08	25.0	0.0454	0.0218	5.9
..do....	..do....	1.59	19.1	0.0372	0.0235	2.7
..do....	..do....	1.61	19.4	0.0363	0.0225	.....
	July 11	4.05	48.5	0.0593	0.0146	11.0
	July 16	3.71	44.5	0.0556	0.0149	14.8
Average.....		2.50	30.0	0.0487	0.0206	7.4
<i>Artemisia tridentata</i> Nutt.....	June 9	1.58	19.0	0.0195	0.0124	1.9
	June 15	1.90	22.8	0.0247	0.0130	2.9
..do....	..do....	2.36	28.4	0.0232	0.0098	3.6
	June 17	1.20	14.4	0.0198	0.0165	2.6
..do....	..do....	1.24	14.9	0.0203	0.0164	2.5
	July 11	5.96	71.1	.....	.....	6.3
	July 16	6.11	72.9	0.0206	0.0033	5.3
	July 17	2.73	32.8	0.0237	0.0086	3.8
	July 28	3.05	36.5	0.0218	0.0071	3.9
Average.....		2.90	34.8	0.0217	0.0109	3.6
<i>Atriplex confertifolia</i> (Torr.) S. Wats.	June 15	4.32	51.7	0.0813	0.0188	26.8
<i>Chrysothamnus</i> sp.....	June 17	1.14	13.7	0.0228	0.0205	2.5
<i>Chrysothamnus marianus</i> Rydb.....	June 15	1.68	20.2	0.0235	0.0140	2.5
<i>Tetradymia glabrata</i> A. Gray.....	..do....	1.89	22.7	0.0321	0.0169	8.6
<i>Tetradymia spinosa</i> H. & A.....	June 17	1.41	17.0	0.0353	0.0250	2.4
<i>Sarcobatus vermiculatus</i> (Hook.) Torr.....	June 15	1.96	23.6	0.0453	0.0230	3.7
	July 11	2.44	29.3	0.0486	0.0199	8.8
Average.....		2.20	26.5	0.0469	0.0215	6.3
HERBS						
<i>Amsinckia tessellata</i> A. Gray.....	June 9	1.19	14.3	0.0232	0.0195	.....
<i>Camelina microcarpa</i> Andr.....	..do....	1.28	15.4	0.0284	0.0222	5.0
<i>Cleome serrulata</i> Pursh.....	June 17	1.06	12.8	0.0176	0.0166	0.9
..do....	..do....	1.17	14.1	0.0190	0.0161	1.3
Average.....		1.12	13.5	0.0183	0.0164	1.1
<i>Eurotia lanata</i> (Pursh) Moq.....	June 17	1.43	17.2	0.0270	0.0189	2.3
<i>Lepidium pubicarpum</i> A. Nels. ....	June 9	1.29	15.5	0.0241	0.0188	2.0
<i>Pogonobergii</i> Vasey.....	June 17	1.48	17.8	0.0204	0.0136	3.1
<i>Sidnion hystrix</i> (Nutt.) Smith.....	..do....	1.63	19.6	0.0280	0.0172	3.4
<i>Sophia filipes</i> (A. Gray) Heller....	June 9	1.55	18.6	0.0218	0.0141	2.1

The type species of the association is interesting in that it may occur sporadically in any other association. Our predecessors (24) have shown that in the typical *Kochia* association the upper foot of soil is usually free from injurious quantities of salts but that saline substances are extremely concentrated in the second, and especially in the third foot and at lower levels. Further details are given elsewhere (24).

Table VI may be consulted for the constants of the species of the *Kochia* association.

#### SHADSCALE ASSOCIATION

The permanent vegetation of the shadscale association is, practically speaking, limited to the single ligneous species *Atriplex confertifolia*, which, as a component of vegetation, is one of the most important species of the Great Basin. It is abundant on mountains above the Bonneville level, and extends below the association of which it is the type, to form a prominent element of the greasewood-shadscale association on the lower slopes of the valley and on the ridges which are interspersed among the salt flats along the southern shore of the Great Salt Lake.

Where the gradient is so slight that the land appears practically level, the shadscale is the only conspicuous ligneous species. The association is, however, diversified in places by small washes in which the number of woody species is larger. There are also a few ephemeral and perennial herbs.

In the summary of the investigations on the physical environment of this association, we note (24) that it is usually characterized by "(1) a soil of finer texture, having a higher moisture equivalent than in sagebrush land; (2) a deficit in midsummer of moisture available for plant growth; (3) a high salt content of the soil below the depth of 1 or 2 feet; and (4) as compared with land occupied by the *Kochia* association, a somewhat lighter and more gravelly texture in the first foot and a much more uneven surface—conditions which probably result in better penetration and, hence, in a larger seasonal total of water available for plant growth than on *Kochia* land."

It has seemed desirable to divide our determinations into two series which can not, however, be sharply separated—the typical shadscale association and the washes through the shadscale association.

#### THE TYPICAL SHADSCALE ASSOCIATION

The constants for the species of this association appear in Table VII.

TABLE VII.—Physicochemical constants for species of the typical shadscale association

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
HALF SHRUBS						
<i>Atriplex confertifolia</i> (Torr.) Wats..	1920.					
	June 2	2.74	32.9	0.0586	0.0214	.....
	...do.....	2.36	28.3	.0535	.0227	10.2
	July 23	4.82	57.6	.0727	.0150	...
	...do.....	4.65	55.7	.0700	.0150	25.0
Average.....		3.64	43.6	.0637	.0185	17.6

Table VII.—Physicochemical constants for species of the typical shadscale association—Continued

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity, to depression, K/ $\Delta$ .	Chlorids per liter. Cl.
GRASSES						
	1920.					
<i>Bromus tectorum</i> L.....	June 2	1.52	18.3	0.0221	0.0145	2.2
<i>Bromus tectorum nudus</i> Klett & Richt.....	May 28	1.20	14.4	.0215	.0179	.....
	June 2	1.05	12.7	.0244	.0232	2.5
Average.....		1.13	13.6	.0230	.0206	2.5
<i>Sitanion hystrix</i> (Nutt.) Smith.....	May 31	1.78	21.4	.0292	.0164	.....
	June 2	1.70	20.4	.0285	.0168	.....
Average.....		1.74	20.9	.0289	.0166	.....
<i>Sitanion jubatum</i> Smith.....	May 28	1.30	15.6	.0261	.0201	.....
HERBS, OTHER THAN GRASSES						
<i>Amsinckia tessellata</i> A. Gray.....	June 1	0.99	12.0	.0215	.0217	.....
<i>Cheirinia repanda</i> (L.) Link.....	May 31	1.28	15.4	.0283	.0221	.....
<i>Lappula occidentalis</i> (S. Wats.) Greene.....	do.	1.09	13.1	.0243	.0223	.....
<i>Sophia filipes</i> (A. Gray) Heller ....	June 1	1.42	17.0	.0190	.0134	.....
<i>Sophia viscosa</i> Rydb.....	May 31	1.40	16.8	.0242	.0173	.....
<i>Sphaerostigma tortum</i> (Lev.) A. Nels.....	do.	1.05	12.6	.0207	.0197	.....
<i>Salsola pestifer</i> A. Nels.....	June 5	1.43	17.2	.0386	.0270	2.1

## WASHES THROUGH THE SHADSCALE ASSOCIATION

The constants for the individual species of the washes through the shadscale association appear in Table VIII.

TABLE VIII.—Physicochemical constants for species of the washes through the shadscale association

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter. Cl.
SHRUBS AND HALF SHRUBS						
	1920.					
<i>Artemisia tridentata</i> Nutt.....	May 31	1.33	16.0	0.0205	0.0154	.....
	June 1	1.39	16.7	.0197	.0142	.....
Average.....		1.36	16.4	.0201	.0148	.....
<i>Chrysothamnus</i> sp.....	May 28	1.23	14.8	.0192	.0157	.....
Do.....	do.	1.13	13.6	.0193	.0171	.....
Do.....	May 31	1.45	17.4	.0219	.0151	.....
Do.....	do.	1.15	13.8	.0224	.0195	.....
Do.....	July 23	1.34	16.1	.0222	.0166	2.2
<i>Sarcobatus vermiculatus</i> (Hook.) Torr.....	May 31	1.73	20.9	.0435	.0251	4.9
	June 1	1.68	20.2	.0423	.0252	.....
Average.....		1.71	20.6	.0429	.0251	4.9
<i>Tetradymia glabrata</i> A. Gray.....	June 1	1.37	16.5	.0282	.0205	.....
<i>Kochia vestita</i> S. Wats.....	June 2	1.57	18.9	.0371	.0236	.....
HERBS						
<i>Iva axillaris</i> Marsh.....	June 1	1.13	13.6	.0262	.0232	5.5
<i>Marrubium vulgare</i> L.....	May 31	1.23	14.7	.0243	.0198	.....
<i>Nicotiana attenuata</i> Torr.....	July 23	1.37	16.5	.0208	.0152	3.1
<i>Rubia tinctorum</i> L.....	May 28	0.89	10.7			.....

## GREASEWOOD-SHADSCALE ASSOCIATION

This association, in which the two dominant species are the greasewood, *Sarcobatus vermiculatus*, and the shadscale, *Atriplex confertifolia*, occupy ridges which diversify the broad mud flats or salt flats which skirt the southern shore of the Great Salt Lake.

According to the observations of Kearney, Briggs, Shantz, McLane, and Peimeisel (24), it occupies all areas where the water table is sufficiently high to make moist soil accessible to the deep-rooting greasewood and where at the same time the upper foot or two are sufficiently dry to permit the growth of the shadscale. Where the water table is too low, as on the higher outwash slopes, this association gives way to the shadscale association. Where the water table approaches the surface, the greasewood is associated with *Allenrolfea* instead of with shadscale, or else the greasewood is replaced by the more salt-tolerant *Allenrolfea* and *Salicornia*.

In typical areas the two dominant species intermingle in approximately equal numbers, but on the higher slopes shadscale is the more abundant while on the lower fringes of the association the greasewood is more abundant. In the lowest zone both the greasewood and the shadscale are apt to be dwarfed.

In classifying our determinations it has been difficult to decide in a few instances whether individual collections should be referred to this association or to the salt flat communities.

TABLE IX.—Physicochemical constants for species of the greasewood-shadscale association

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS						
<i>Sarcobatus vermiculatus</i> (Hook.)	1920.					
Torr.....	May 29	2.30	27.6	0.0589	0.0256	.....
...do.....	June 8	1.89	22.7	.0421	.0222	.....
...do.....	June 12	2.12	25.5	.0482	.0227	6.0
...do.....	June 18	2.21	26.5	.0508	.0230	7.5
...do.....	June 18	2.94	35.3	.0648	.0220	14.3
...do.....	July 11	2.34	28.1	.0507	.0216	7.0
...do.....	July 16	2.50	29.0	.0518	.0208	8.8
...do.....	July 16	3.32	39.8	.0626	.0188	12.3
Average.....	July 16	2.32	27.9	.0502	.0216	4.8
		2.44	29.3	.0533	.0220	8.7
<i>Atriplex confertifolia</i> (Torr.) S.						
Wats.....	May 29	2.60	31.2	.0589	.0228	.....
...do.....	June 12	3.22	38.6	.0711	.0220	30.2
...do.....	June 18	3.29	38.0	.0636	.0193	19.5
...do.....	July 16	3.21	38.5	.0672	.0209	21.2
...do.....	July 18	6.22	74.2	.0970	.0156	39.5
...do.....	July 27	10.00	118.5	.1235	.0123	64.0
Average.....	July 27	12.99	153.1	.1292	.0099	64.4
		5.93	70.3	.0872	.0175	39.8

TABLE IX.—Physicochemical constants for species of the greasewood-shadscale association—Continued

Growth form and species.	Date.	Freezing point depression, Δ.	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/Δ.	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS—CON.						
1920.						
<i>Atriplex nuttallii</i> S. Wats. ....	May 29	3.17	38.0	0.0727	0.0229	.....
do. ....	do. ....	2.98	35.8	0.0612	0.0205	.....
June 8	June 8	3.46	41.5	0.0725	0.0209	27.6
June 18	June 18	3.39	40.6	0.0610	0.0180	22.6
July 27	July 27	14.40	169.3	0.1249	0.0086	93.1
Average. ....		5.48	65.0	0.0785	0.0182	47.8
<i>Kochia vestita</i> S. Wats. ....	June 18	2.48	29.7	0.0511	0.0206	6.4
July 11	July 11	3.35	40.2	0.0604	0.0180	4.4
July 18	July 18	3.84	46.0	0.0582	0.0151	8.4
July 27	July 27	3.93	47.0	0.0662	0.0168	14.4
Average. ....		3.40	40.7	0.0590	0.0176	8.4
<i>Allenrolfea occidentalis</i> (S. Wats.) Kuntze. ....	May 29	1.87	22.5	0.0468	0.0250	.....
June 12	June 12	3.69	44.2	0.0695	0.0188	23.5
June 18	June 18	3.32	39.8	0.0658	0.0198	17.4
do. ....	do. ....	3.31	39.7	0.0648	0.0196	15.4
July 11	July 11	5.17	61.8	0.0700	0.0135	16.5
July 16	July 16	3.95	47.3	0.0663	0.0167	14.9
Average. ....		3.55	42.6	0.0639	0.0189	17.5
<i>Dondia torreyana</i> (S. Wats.) Standley. ....	June 12	3.68	44.1	0.0729	0.0198	22.0
June 18	June 18	2.78	33.4	0.0581	0.0208	11.5
do. ....	do. ....	2.79	33.5	0.0606	0.0217	13.0
do. ....	do. ....	2.55	30.6	0.0573	0.0225	10.9
July 16	July 16	3.11	37.4	0.0575	0.0184	12.3
July 18	July 18	3.25	38.9	0.0605	0.0186	12.8
July 27	July 27	3.85	46.1	0.0619	0.0160	13.6
Average. ....		3.14	37.7	0.0612	0.0197	13.7
<i>Artemisia tridentata</i> Nutt. ....	May 29	1.24	14.9	0.0191	0.0152	.....
June 18	June 18	1.56	18.7	0.0211	0.0135	2.6
Average. ....		1.40	16.8	0.0201	0.0144	2.6
<i>Artemisia spinescens</i> D. C. Eat. ....	May 29	1.26	15.2	0.0228	0.0180	.....
<i>Tetradymia glabrata</i> A. Gray. ....	June 18	1.93	23.2	0.0354	0.0183	9.5
<i>Tetradymia nuttallii</i> T. & G. ....	May 28	1.62	19.5	0.0308	0.0190	.....
<i>Tetradymia spinosa</i> H. & A. ....	May 29	1.49	18.0	0.0290	0.0194	.....
<i>Chrysothamnus</i> sp. ....	do. ....	1.46	17.5	0.0209	0.0143	.....
<i>Chrysothamnus nauseosus</i> (Pursh) Britton. ....	July 14	1.66	20.0	0.0261	0.0157	.....
PERENNIAL HERBS						
<i>Oreocarya</i> sp. ....	May 29	0.88	10.6	0.0178	0.0203	.....
ANNUAL HERBS						
<i>Cheirinia</i> sp. ....	May 29	1.02	12.2	0.0194	0.0191	.....
Do. ....	May 28	0.94	11.3	0.0161	0.0172	.....
<i>Cheirinia asperrima</i> (Greene) Rydb. ....	May 29	0.92	11.1	0.0164	0.0177	1.7
<i>Lepidium scopulorum</i> M. E. Jones. ....	do. ....	1.15	13.9	0.0257	0.0223	.....
<i>Thelypodium elegans</i> Jones. ....	do. ....	0.89	10.8	0.0111	0.0211	.....
<i>Thelypodium macropetalum</i> Rydb. ....	do. ....	0.88	10.6	0.0211	0.0241	.....

Few species are found in addition to the two type species, *Sarcobatus* and *Atriplex confertifolia*, and the half shrubs, *Atriplex nuttallii*, *Kochia vestita*, *Dondia torreyana* and in the transition zones *Allenrolfea*. Other shrubs are extremely rare, and those included here are given because of the interest which attaches to determinations based on species which are out of their natural range.

Herbaceous species are practically limited to annuals which complete their growth early in the season.

Table IX contains the constants for the individual species of the greasewood-shadscale association.

#### GRASS-FLAT COMMUNITIES

The grass flats, which occupy the lowest areas of the region under consideration with the possible exception of the salt flats, are abundantly supplied with moisture. This is in part supplied by springs and seepage, which vary greatly in salinity. The investigations of our predecessors in this field indicate that "the soils are characterized by (1) a high moisture-holding capacity, ascribable partly to the fine texture and partly to the large quantity of organic matter present, (2) the presence near the surface and usually throughout the summer of moisture available for growth (above the wilting coefficient), and (3) a salt content sufficiently high to be injurious to many crop plants."

The great diversity in conditions may be illustrated by determinations on the spring water in two different localities. Determinations on the water from the springs at the northern end of the Stansbury Mountains, and from the drainage channels from these springs, are given below (p. 913). These may be compared with one sample taken from a small slough in the lower part of the valley between Grantsville and Garfield which gave, on July 27,  $\Delta = 0.07$ ,  $P = 0.84$ ,  $K = .0017$ ,  $K\Delta = .0251$ ,  $Cl = 0.3$ .

Kearney and his associates point out (24) that while the vegetation of the grass flats is for the most part distinctly halophytic, there are limited areas around springs where the vegetation resembles that of the ordinary nonsaline meadow. The vegetation shows considerable diversity, and several subdivisions may ultimately be recognized. A precise classification of the vegetation must, therefore, await further investigation. The transition between the grass-flat communities and the greasewood-shadscale association and the salt-flat communities is not sharp.

Our series of determinations for the grass-flat species is inadequate. This is in part due to the fact that the grass land near Grantsville has been intensively grazed and mowed, and in part to the fact that it is extremely difficult to obtain samples of some of the grasses. The leaves of *Distichlis spicata* are often covered with salt, and to remove this without influencing the concentration of the leaf-tissue fluids is very difficult.

The determinations given must, therefore, be considered as preliminary notes on some of the species rather than as an adequate treatment of the vegetation. Unless otherwise stated, the determinations are based on collections near the southern shores of the Great Salt Lake.

Table X gives the constants for species other than the grasses. Because of the difficulty of working with the latter, it has been necessary to include determinations made in places other than Tooele Valley.



TABLE X.—Physicochemical constants for species other than grasses in the grass-flat communities

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS						
<i>Sarcobatus vermiculatus</i> (Hook.) Torr.	1920. June 18	2.10	25.2	0.0499	0.0238	7.7
<i>Atriplex nuttallii</i> S. Wats.	do.	3.09	37.1	0.0587	0.0190	17.1
<i>Allenrolfea occidentalis</i> (S. Wats.) Kuntze.	do.	3.11	37.2	0.0617	0.0198	17.5
	July 27	3.70	44.4	0.0700	0.0186	21.8
	do.	3.43	41.1	0.0675	0.0197	25.0
	do.	3.41	40.9	0.0672	0.0197	.....
	do.	3.56	42.6	0.0600	0.0194	19.1
	do.	3.21	38.5	0.0652	0.0203	14.6
Average		3.40	40.8	0.0667	0.0196	19.6
HERBS, OTHER THAN GRASSES						
<i>Salicornia rubra</i> A. Nels.	July 27	3.24	38.9	0.0615	0.0190	27.3
<i>Atriplex rosea</i> L.	June 18	2.02	24.3	0.0416	0.0206	9.5
	July 1	3.24	38.9	0.0601	0.0185	22.1
Average		2.63	31.6	0.0509	0.0195	15.8
<i>Grindelia squarrosa</i> (Pursh) Dunal.	July 27	1.47	17.6	0.0324	0.0220	5.0
MARSH HERBS						
<i>Eleocharis</i> sp.	do.	1.07	12.9	0.0251	0.0235	5.1
<i>Juncus balticus</i> Willd.	do.	1.36	16.4	0.0290	0.0213	7.1
<i>Scirpus olneyi</i> A. Gray.	June 12	1.50	18.0	0.0367	0.0244	8.8
	July 11	1.34	16.4	0.0322	0.0240	7.5
	July 27	1.16	14.0	0.0316	0.0273	4.7
Average		1.33	16.0	0.0335	0.0252	7.0
<i>Triglochin maritima</i> L.	June 12	2.64	31.6	0.0535	0.0203	21.1
	June 18	2.64	31.6	0.0528	0.0200	19.7
Average		2.64	31.6	0.0532	0.0202	20.4

TABLE XI.—Physicochemical constants for the grasses in the grass-flat communities

Name.	Date.	Depression of freezing point, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter, Cl.
1920.						
<i>Sporobolus airoides</i> Torr.	July 27	2.82	33.9	0.0441	0.0156	15.2
	June 28	1.90	22.8	0.0338	0.0178	7.0
	July 17	2.45	29.4	0.0396	0.0162	11.9
<i>Distichlis spicata</i> (L.) Greene	July 5	2.88	34.5	.....	.....	13.4
	do.	3.63	40.5	0.0520	0.0143	20.0
1921						
	June 20,	2.94	35.3	0.0371	0.0126	.....
	June 29	1.64	19.8	0.0284	0.0172	7.9
<i>Hilaria jamesii</i> (Torr.) Benth.	do.	1.07	12.9	0.0172	0.0161	2.1

Table XI gives the constants for the sap properties of the grasses.

*SPOROBOLUS AIROIDES* TORR.—The first determination of July 27 was made on material taken in Tooele Valley. The sample of June 28 was taken on the small sand dunes southeast of Pavant Butte in the Sevier Desert. This species also occupies low depressions on the leeward side of sand dunes on the eastern edge of the sterile portions of the Great Salt Lake Desert at Knolls, where it was taken on July 17.

*DISTICHLIS SPICATA* (L.) GREENE.—It is not known just how large a concentration of soil solution *D. spicata* will tolerate. Kearney and his associates noted that the salt concentration is higher in the soil under the salt-grass community than under that occupied by the *Sporobolus-Chrysothamnus* community. The writers' notes show that where the drainage water from the thermal springs at the northern end of the Stansbury Mountains is abundant, *D. spicata* and *Salicornia utahensis* occur in association. A reading on the water indicates an osmotic concentration of about 21.6 atmospheres and a conductivity of 0.046 mho.

For actual determinations on the tissue fluids of *Distichlis spicata* we must turn to collections made elsewhere than in Tooele Valley. A sample was taken on the low grounds east of the Ice Spring Craters lava field, Sevier Desert, on July 5.

At the bottom of the Terrace Crater, Ice Spring Craters, Sevier Desert, is a small plot of grass which has apparently remained in about its present condition since it was first noted by the geologists of the Wheeler survey. It is a pure stand of *Distichlis spicata*. The plot is not much higher than the level of the ground water in the ancient lava vent, which has been shown to be rather saline (8). The sample taken at midday on July 5 (the second sample of that date) was possibly slightly wilted and with a trace of excreted salt on the surface of the leaves. Repetition of this collection on July 20, 1921, gave somewhat lower values.

These determinations indicate a rather high concentration, including an abundance of chlorids, in the leaf-tissue fluids of the salt grass when growing in its typical halophytic habitats. When growing where salts are less abundant, as on the sandy plain which represents a portion of the ancient delta of the Sevier River (sample of June 29), the plant shows a much lower concentration.

The values for osmotic concentration and electrical conductivity are only about half as large as those which were found when the species was growing in highly saline areas. The chlorid content is only about one-fourth as high. One of these lacks a conductivity measurement. That *Distichlis spicata* has an inherently higher concentration than some other grass species, and that the concentration is not determined solely by the environment is shown by comparison with determinations made on *Hilaria jamesii* with which it was immediately associated, (June 20), on the ancient delta of the Sevier River. We may note that *H. jamesii* contains only about half the chlorids found in *D. spicata* in association with it.

#### SALT-FLAT COMMUNITIES

The most highly saline conditions of the region are encountered in the great mud flats which cover an area of many square miles along the southern shore of the Great Salt Lake. These flats are diversified by low ridges, which have already been considered in the discussion of the greasewood-shadscale association. The flats are so gently sloping that they appear level. In drier periods the grit of salt can be felt as one

walks across them, and when viewed from such a distance that the scattered hummocks of vegetation can not be seen, they are gleaming white.

These flats are practically devoid of vegetation except for the two extreme halophytes, *Allenrolfea occidentalis* and *Salicornia utahensis*, both half shrubs. Annual species may start growth, and even flower on these hummocks, but this is due only to their capacity for rapid growth, which may be considerably advanced before the salts which have been leached out of the upper layers of soil by the spring rains again become highly concentrated at the surface. Probably any of the annual species which are reported from the ridges in the flats (see greasewood-shadscale association) may begin, or perhaps even occasionally complete, a very dwarfed development on the low hummocks which constitute the vegetated fraction of the salt flats. The most characteristic of these species is the minute *Capsella elliptica* Meyer, which was found in countless numbers on the lower portions of the ridges and even on the hummocks. These plants were, however, practically all matured at the time of our first visit, on May 28. Thus they must be characterized by early germination and extremely rapid development.

Water is generally found at a relatively short distance below the surface. This may differ greatly in concentration from place to place, as is shown by the following determinations (Table XII) based on samples from shallow borings.

TABLE XII.—Physicochemical constants for spring, ground, and surface water, Tooele Valley, Utah

Number of sample.	Depth to water, in inches.	Chief constituent of vegetation.	Freezing point depression, $\Delta$ .	Atmospheres, osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter, Cl.
1	Less than 12.....	Allenrolfea and Salicornia.	2.75	33.1	0.0608	0.0220	.....
2	About 12.....	do.....	2.52	30.4	.0597	.0236	24.5
3	About 26.....	do.....	3.21	38.4	.0704	.0219	31.4
4	About 16.....	Sterile.....	3.79	45.4	.0750	.0197	37.0
5	Unrecorded.....	do.....	8.01	95.2	.1108	.0138	72.5
6	Thermal springs.....	.....	1.94	23.3	.0689	.0355	19.3
7	Drainage channels.....	.....	2.51	30.2	.0470	.0187	24.9
8	.....do.....	.....	1.79	21.6	.0462	.0257	30.4
9	.....do.....	.....	1.79	21.6	.0476	.0265	17.7
10	Spring.....	.....	0.67	8.0	.0203	.0305	6.2
11	Drainage from spring.....	.....	0.68	8.1	.0211	.0312	6.2
12	Surface water.....	.....	4.03	48.3	.0865	.0214	38.5

Sample 1 was taken from a small hummock on the generally sterile mud flats near Bermester. Samples 2-5 were taken within a narrow radius on the highly saline, and largely sterile, mud flats near the warm springs at the foot of the Stansbury Mountains northwest of Grantsville. These thermal springs differ in temperature and salinity. Sample 6 was taken from the deepest of the three springs, on June 18. Water from the drainage channels from this group of springs was taken in two places on June 18 (samples 7-8) and again on July 1 (sample 9).

There are large springs near the northern end of the Stansbury Mountains, which have small run-off streams which feed limited grass-flat areas, but their waters are soon lost in the sterile mud flats. The concentration of the water of these springs has not been studied in detail, but is much less than that of the ground water as noted above. Sample 10 was taken from a large spring at the northern end of the Stansbury Mountains. Sample 11 was collected from the drainage channel from this spring on June 12. These values show a relatively low concentration, as compared with those given above, but the water soon reaches high concentration through evaporation. This is shown by the results for sample 12 collected on the same date (June 12) from a depression in the mud flats, and which had probably in part been derived from these same springs. The results indicate that the concentration of the ground water varies enormously in relatively short distances, and suggest that the sheet of water must be derived in large part from higher levels. Near Bermester there are calcareous rings which indicate the presence of active springs at an earlier period.

The constants show the great variations of concentration to which the species which struggle for existence in this region may be subjected.

The determinations on the plant species are given in Table XIII.

TABLE XIII.—Physicochemical constants for species of the salt flats

Growth form and species.	Date.	Freezing point depression, $\Delta$ .	Atmospheres osmotic concentration, P.	Specific electrical conductivity, K.	Ratio, conductivity to depression, K/ $\Delta$ .	Chlorids per liter, Cl.
SHRUBS AND HALF SHRUBS						
<i>Allenrolfea occidentalis</i> (S. Wats.) Kuntze.....	1920. May 28	2.96	35.6	0.0625	0.0212	.....
	June 8	2.45	29.4	.0526	.0430	14.7
	June 12	4.26	51.0	.0808	.0189	25.0
	June 18	4.06	48.6	.0689	.0170	26.1
	July 1	4.27	51.1	.0797	.0186	25.7
Average.....		3.60	43.1	.0689	.0238	22.9
<i>Salicornia utahensis</i> Tidest.....	June 8	3.07	36.8	.0658	.0214	25.5
	June 12	3.66	43.9	.0783	.0213	37.1
	June 18	3.56	42.7	.0686	.0192	30.4
	...do....	3.49	41.8	.0664	.0190	30.3
	...do....	4.14	49.8	.0773	.0187	36.1
	July 1	4.34	51.9	.0820	.0189	25.7
	...do....	3.42	41.0	.0693	.0202	28.4
	...do....	3.25	38.9	.0668	.0205	26.8
Average.....		3.62	43.4	.0718	.0199	30.0
<i>Sarcobatus vermiculatus</i> (Hook.) Torr.....	May 28	2.25	27.1	.0467	.0207	.....
ANNUAL HERBS						
<i>Atriplex hastata</i> L.....	June 8	2.15	25.8	.0479	.0223	.....
	June 11	3.61	43.3	.0659	.0182	24.3
Average.....		2.88	34.6	.0569	.0203	24.3

## DISCUSSION OF RESULTS

Comparison among the various associations may be made in two ways, (a) by the comparison of the average values of the whole series of determinations made in the several associations and (b) by the comparison of constants based on one and the same species when growing in two different associations.

Notwithstanding the very large number of determinations made in our field operations, a fully satisfactory comparison on either basis is not yet possible. Conditions of vital importance to the plant, such as soil moisture, soil salinity, temperature, insolation, and the evaporating power of the air, change rapidly with the march of the season. Thus two determinations on the same species in two different associations are not directly comparable unless synchronously made. The great labor of securing the samples and carrying out the measurements renders this ideal practically impossible of attainment. Thus the comparison here made must be regarded as tentative.

## COMPARISON OF THE VARIOUS ASSOCIATIONS OF TOOELE VALLEY AMONG THEMSELVES

The averages of the various constants for the habitats discussed in the preceding pages appear in Table XIV.

Considering first the mean values of freezing point lowering in degrees,  $\Delta$ , and osmotic concentration in atmospheres,  $P$ , we may note first of all that in every instance the osmotic concentration of ligneous plants is higher than that of herbaceous plants. This result is in agreement with that derived from earlier studies in the more mesophytic region of Long Island, N. Y. (11), in the Jamaica Montane Rain Forest (17), and in the Arizona deserts (12). In the comparison of the various habitats we must, therefore, direct our attention to the two growth forms separately.

It is clear that for the ligneous plants the osmotic concentration in the sagebrush association is higher than that in the Stansbury Mountains at higher levels. This is true whether we consider the three subdivisions of the sagebrush association separately or class all three together.

The number of ligneous species from the sandhill mixed association is too small to justify a comparison between it and the sagebrush association. The average of the three ligneous species is, however, higher than that of the ligneous plants of the Stansbury Mountains at higher elevations.

The ligneous plants of the *Kochia* association show a higher osmotic concentration than that found in any of the foregoing habitats.

The ligneous species in the shadscale association as a whole seem to show lower concentration than those of the sagebrush association. This is, however, due to the fact that most of the ligneous species recorded as occurring in the shadscale association are found in the washes where they doubtless have a much larger supply of water. The average of the determinations on the shadscale itself is 43.6 atmospheres, a value far higher than that of any of the preceding. Even higher values for the leaves of the shadscale have been demonstrated elsewhere and probably would be found here if determinations were made at the period of maximum dryness.

TABLE XIV.—Average values of the physicochemical constants for the various plant associations

Habitat.	Mean depression of freezing point, Δ.	Mean atmospheric osmotic concentration, P.	Mean specific electrical conductivity, K.	Mean ratio, conductivity to depression, K/Δ.	Mean chlorid per liter, Cl.
Stansbury Mountains at higher altitudes (Table I):					
Ligneous.....	1.419	17.06	0.01225	0.00904	0.68
Herbaceous.....	1.094	13.17	0.01726	0.01674	1.42
Sagebrush association <sup>a</sup> :					
Ligneous.....	1.832	22.01	0.02433	0.01333	2.65
Herbaceous.....	1.165	14.01	0.02132	0.01884	2.83
Sagebrush association of the foothills canyons (Table II):					
Ligneous.....	1.625	19.50	0.01535	0.01005	0.88
Herbaceous.....	1.166	14.01	0.02209	0.01988	3.58
Washes through sagebrush association (Table III):					
Ligneous.....	2.070	24.88	0.02704	0.01308	3.31
Herbaceous.....	1.359	16.33	0.02136	0.01563	2.76
Sagebrush association, alluvial fans (Table IV):					
Ligneous.....	1.723	20.70	0.02552	0.01459	2.77
Herbaceous.....	1.124	13.53	0.02073	0.01871	2.36
Sand-hill mixed association (Table V):					
Ligneous.....	1.713	20.63	0.02503	0.01470	2.90
Herbaceous.....	1.101	13.24	0.02096	0.01934	1.75
Kochia association (Table VI):					
Ligneous.....	2.255	27.08	0.03904	0.01853	7.53
Herbaceous.....	1.371	16.49	0.02390	0.01759	2.71
Shadscale association: <sup>a</sup>					
Ligneous.....	1.546	18.58	0.02836	0.01845	5.88
Herbaceous.....	1.207	14.51	0.02479	0.02017	3.57
Typical shadscale association (Table VII):					
Ligneous.....	1.866	22.40	0.03276	0.01806	7.43
Herbaceous.....	1.237	14.87	0.02523	0.02050	2.10
Washes through shadscale association (Table VIII):					
Ligneous.....	1.368	16.46	0.02592	0.01867	3.55
Herbaceous.....	1.155	13.88	0.02377	0.01940	4.30
Greasewood-shadscale association (Table IX):					
Ligneous.....	2.674	31.98	0.04525	0.01792	18.50
Herbaceous.....	0.954	11.50	0.01942	0.02012	1.70
Grass-flat communities (Table X):					
Ligneous.....	2.863	34.37	0.05843	0.02080	14.80
Herbaceous.....	1.963	23.57	0.04080	0.02153	12.53
Salt-flat communities (Table XIII):					
Ligneous.....	3.157	37.87	0.06247	0.02143	26.45
Herbaceous.....	2.880	34.60	0.05690	0.02030	24.30

<sup>a</sup> These averages have been obtained by merely combining the totals for the subhabitats given below. They have not been recalculated from new species means in the cases in which the same species is found in two of the subhabitats.

The ligneous plants of the greasewood-shadscale association, the grass-flat communities and the salt-flat communities average 32 to 38 atmospheres. These figures, which must be regarded as approximate merely, show that the osmotic concentration found in the native vegetation of these habitats is far higher than that which is found in the tissue fluids of cultivated plants. Thus the physicochemical characteristics of the tissue fluids of the native vegetation are such as to indicate that few if any cultivated plants have osmotic concentration in their tissue fluids which would suit them for prolonged growth in such an environment as that presented by the shadscale association, the greasewood-shadscale association, the grass-flat, and the salt-flat communities as they exist in Tooele Valley. This does not necessarily imply that such habitats could not be suited for crop growth by proper irrigation under conditions permitting the removal of the salts from the soil by leeching and drainage.

Comparing the mean values of specific electrical conductivity in ligneous and herbaceous plants, it appears that in every unit of classification except the Stansbury Mountains at higher altitudes and the sagebrush association of the foothills canyons, the conductivity is higher in the ligneous than in the herbaceous plants.

This result is in apparent disagreement with the findings of an earlier paper (20) in which it was shown that the specific electrical conductivity of ligneous plants is lower than that of herbaceous forms.

It must be remembered, however, that in the desert proper we are comparing herbaceous species of a very ephemeral nature with ligneous species which, to a greater or less extent, remain active throughout the season. Many of the latter are typical halophytes. Thus it might be expected that the ligneous plants would absorb and retain in solution larger quantities of salts than the herbaceous forms.

In the Stansbury Mountains at higher elevations and in the sagebrush association of the foothills canyons where the conditions are more mesophytic the conductivity of herbaceous plants is higher than that of ligneous plants, just as has been found to be the case in determinations made on Long Island.

The average value of the ratio of specific electrical conductivity to freezing point depression  $K/\Delta$  is higher in herbaceous than in ligneous plants in all the units of classification of the vegetation, with the exception of the constants based on the wholly inadequate series of determinations on herbaceous species in the *Kochia* association and in the salt flats. This result is in full agreement with the writers' earlier findings.

Turning back to the osmotic concentrations of the herbaceous species, it is clear that there is considerable irregularity in the values for the associations higher than the grass flats and the salt flats. It is difficult to be certain of the same graduation in the magnitudes of osmotic concentration as has been demonstrated for the ligneous species. It must not be forgotten, however, that we are here dealing largely with ephemeral species, which die down before the conditions become very severe. The average osmotic concentrations for the grass flats and salt flats are conspicuously higher than those found in the other associations.

The chlorid content of the species of the greasewood-shadscale association, of the grass-flat communities, and of the salt flats is conspicuously higher than that of any other habitat. The series of analyses is not sufficiently large to render further discussion desirable at this time.

COMPARISON OF CERTAIN OF THE DOMINANT OR TYPE INDICATOR SPECIES  
OF THE REGION CONSIDERED

In the preceding section we discussed the average values of the constants for the various plant associations, pointing out the possible sources of error in such a method of comparison and referring to the desirability of a comparison based on the individual species constants.

Limitations of space preclude the making of such a detailed comparison in this place, and it is reserved for publication at a later time.

The chief indicator species of the region, and a detailed discussion of the physical conditions of the habitats in which they occur, may be found in the lists of species discussed by our predecessors (24). Their data, taken in combination with those presented in the tables of this paper, make possible for the reader a comparison of the individual species among themselves.

COMPARISON OF TOOELE VALLEY WITH CERTAIN OTHER REGIONS WHICH  
HAVE BEEN INVESTIGATED

Those who have read the various papers on the sap properties of the native vegetation of other regions, cited in the introduction of this paper, will note that the osmotic concentrations reported in the Utah habitats are very high as compared with those generally found elsewhere. The only comparable values are those found by Cavara (1) in plants growing along the margins of salt-concentrating basins, by Fitting (5) in the extreme conditions of North African deserts, by Harris, Lawrence, and Gortner (12) in the most saline portions of the Arizona deserts, by Harris and Lawrence (15) in the more saline regions of the Jamaican coastal deserts, and by Harris and Lawrence (16) in the mangrove swamp. These results are in full agreement with the theory that the physicochemical properties of the leaf-tissue fluids furnishes an excellent index of the physical and chemical conditions of the soil, and lends strength to the conclusion that only agricultural plants of extremely short-growth periods, such as would enable them to mature during the period of more abundant soil moisture and lower salinity of the surface layers of soil, or which are capable of developing high osmotic concentration and electrical conductivity of their tissue fluids, may be expected to thrive under the more extreme conditions, unless these are modified by irrigation.

A more detailed comparison of the constants of the plants of this region with those of other regions would be of interest from the ecological rather than from the agricultural side, and will not be attempted here. An investigation of other Utah and Arizona desert regions is in progress, and further discussion may await the completion of these more extensive studies.

## BEARING OF THE RESULTS ON THE PROBLEM OF CROP PRODUCTION

In discussing the results of their study of the botanical composition of the natural vegetation of Tooele Valley in relation to the physical factors of the soil, Kearney, Briggs, Shantz, McLane, and Piemeisel (24) point out that within limits, which are defined, "... All important variations in the character of the soil are clearly expressed in the appearance and botanical composition of the plant covering. In other



words, the principal types of vegetation, where typically developed, are reliable indicators of the physical conditions of the environment."

In summarizing their observations on the attempts, some of them highly successful and others complete failures, to utilize the region for agricultural production, they point out that successful growth of crop plants, with or without irrigation, is so related to the physical and chemical characteristics of the soil, that within limits it can be predicted from the botanical composition of the native vegetation.

Our studies, conducted with the definite purpose of supplementing those of our predecessors, have shown that such physicochemical properties of the plant-tissue fluids as osmotic concentration, specific electrical conductivity, and chlorid content differ from type to type of vegetation, and in such a way as to parallel in a striking manner the findings of our predecessors in this field.

In Tooele Valley inadequacy of soil moisture and a high salt content may combine in various ways to influence the appearance and botanical composition of the vegetation, to determine the physicochemical properties of the native species of which the vegetation is made up, and to limit crop production.

The salts of the soil may conceivably be detrimental to crop production in two ways: First, chemically, through the toxicity of certain of the constituents; second, physically, through the attainment of an osmotic concentration greater than that which can be tolerated by crop plants. The problem of toxicity falls quite outside the scope of this paper.

Quantitative information concerning the concentration of the soil solution as it actually influences the plant in these deserts is not yet available, except for the most extreme conditions as found in the salt flats, where it has been possible to measure the concentration of the ground water, and where the growth of crop plants is obviously impossible.

The large series of physical and chemical measurements made by our predecessors makes it evident, however, that in all the habitats in which any considerable quantity of salt occurs the concentration of the soil solution must become very high when the soil moisture is much reduced by the drying concomitant with the advance of the season.

Investigations on native vegetation, in part published and cited in the introductory section of this paper, but in large part still unpublished, have shown that the osmotic concentration and specific electrical conductivity of the tissue fluids of native vegetation bear an intimate relation to the aridity or salinity of the region. It is but a short—and a very logical—step from this conclusion, which rests upon a large body of facts, to the hypothesis that a fundamental prerequisite for the survival of a plant species, wild or cultivated, in arid regions is a much shortened growth period, a highly efficient water-storage mechanism, or a high osmotic concentration of its tissue fluids.

The generally high osmotic concentration of the tissue fluids of the less ephemeral<sup>6</sup> plant species of the region and the enormous concentrations attained by *Atriplex confertifolia* and *Atriplex nutallii* (21) when growing in such localities is ample evidence for the necessity for very great osmotic concentrations of plant tissue fluids as a prerequisite for perennial growth in most of the region considered.

<sup>6</sup> The lower osmotic concentrations are found in the species which persist for but a brief period during the spring when growth conditions are more favorable than they can continue to be for the maturing of the most agricultural plants, or in the mountains where conditions are suitable only for grazing.

As far as we yet know, few agricultural plants are capable of tolerating salts in such quantities as are present in the soil in certain of these habitats, or of developing the osmotic concentrations which enable them to withdraw water from soil of such texture, salinity, and dryness.

A detailed discussion of the bearing of the results of the present study of natural vegetation on the problem of crop production in the arid and saline regions is limited by the inadequacy of our published knowledge of the physicochemical properties of the tissue fluids of agricultural plants. The pertinent facts are being rapidly supplied by detailed investigations which have been under way for the past three years on the important agricultural plants of the western United States, such as the small grains, the grain sorghums, corn, and cotton.

Studies on the small grains at the Nephi substation of the Utah Agricultural Experiment Station, in a locality formerly occupied by the sagebrush association, have shown that none of the varieties of wheat, oats, barley, spelt, and emmer grown without irrigation under these conditions have tissue fluids with an osmotic concentration as high as that which at comparable seasons of the year characterizes the more permanent native species of the associations occurring at lower levels than the sagebrush.<sup>7</sup>

Crops other than the small grains have been investigated only under irrigation—an artificial condition obviously interfering with the use of indicator plants as a criterion in the selecting of land for crop production.

As grown under irrigation, corn and the grain sorghums show osmotic concentration of the leaf-tissue fluids which are relatively low as compared with the native species discussed in this paper.

Egyptian and Upland cotton when grown with irrigation under the more saline conditions of the Gila River Valley (22) have a much lower osmotic concentration of their leaf-tissue fluids than that of most of the plant species of the more saline regions of the Tooele Valley.

The toxicity to agricultural plants of the salts present in the soil and the incapacity of agricultural plants for developing osmotic concentrations sufficiently high to enable them to withdraw water from the soil, must both be considered in seeking for the physiological explanation of the varying suitability of the various habitats for crop production.

#### SUMMARY AND CONCLUSIONS

The earlier studies of Kearney, Briggs, Shantz, McLane, and Piemeisel (24) have shown that in Tooele Valley, Utah, the plant associations of natural vegetation indicate the conditions of soil moisture and salinity of the land on which they are found and thus afford a basis for the estimation of its suitability for crop production.

The purpose of the present investigation has been a consideration of the physicochemical properties of the leaf-tissue fluids of these indicator plants with a view to determining whether the magnitudes of such constants as osmotic concentration, specific electrical conductivity and chlorid content show a parallelism to the series of soil and vegetation types recognized by our predecessors in this field.

The following salient facts concerning the vegetation may be noted. The statements concerning soils are based upon the findings of our

<sup>7</sup> Comparison of the crop plants with the herbaceous species of the several associations is precluded by the fact that most of the native herbs complete their development and disappear long before the winter grains reach maturity.

predecessors. Those concerning crop production represent their observations as well as our own.

Eight major divisions of the vegetation have been recognized: The Stansbury Mountains at higher altitudes, the sagebrush association, the sand-hill mixed association, the *Kochia* association, the shadscale association, the greasewood-shadscale association, the grass-flat communities, and the salt-flat communities.

With the exception of the Stansbury Mountains at higher altitudes, and to a less extent of the grass-flat and the salt-flat communities, these associations are distinguished primarily by their woody plant species. The herbaceous plants are largely ephemeral, completing their growth during the early spring, while moisture is still available near the surface and while the salts have been to some extent washed out of the superficial layers of soil. These ephemeral species are to some extent common to all the associations. Because of their general distribution, as well as because of the limited period during which they may be identified, they are of less value as indicator plants than the woody species which are active throughout the season. The following generalizations are, therefore, based upon the results for ligneous species.

The sagebrush (*Artemisia tridentata*) association, which occupies the land nearest the mountains and is the highest which is of interest for agricultural (as distinguished from grazing) operations, occurs generally on rather deep soil, of rather light texture, easily permeable to water, and free from large quantities of salts. With the exception of the Stansbury Mountains at higher altitudes and possibly of the sand-hill mixed association, the plants of the sagebrush association have the lowest osmotic concentration, specific electrical conductivity and chlorid content of any of the associations investigated. The average values of these constants are about 22 atmospheres osmotic concentration, 0.024 mho conductivity and 2.65 gm. per liter chlorid content. These values are of about the same order as those found near the end of the season in small grains as grown under dry farm agriculture at Nephi, Utah.<sup>8</sup> The sagebrush land is the locus of the chief dry farming operations of the region.

The *Kochia* (*Kochia vestita*) association which occupies areas lying below the sagebrush zone, is found on land of finer texture, relatively greater impermeability to water, higher moisture-holding capacity, and greater salt content at a small depth below the surface. Sufficient moisture for growth is generally wanting during the summer. Dry farming is precarious, owing to the small depth of soil free from salts. Even under irrigation the relatively impervious nature of the soil might make difficult the washing out of the salts to a depth which would permit profitable crop production. The vegetation is characterized by a higher osmotic concentration, specific electrical conductivity and chlorid content than that of the sagebrush association. When the sagebrush penetrates into the *Kochia* association it is extremely dwarfed, and its tissue fluids may attain an abnormally high osmotic concentration.

The shadscale (*Atriplex confertifolia*) association occupies the outwash slopes below the *Kochia* association. The soil is similar, in the main, to that of the *Kochia* association, but frequently contains much gravel and may become even drier during the summer. Dry farming is hazardous, but where water is available for washing out the salt and supplying

<sup>8</sup> Statement based on unpublished results.

moisture, irrigated crops may be grown. The osmotic concentration, specific electrical conductivity, and chlorid content of the tissue fluids are higher than in any of the preceding associations. The leaf-tissue fluids of *Atriplex confertifolia* may under some conditions develop an osmotic concentration as high as 150 atmospheres, specific electrical conductivity of 0.129 mho, and a chlorid content equivalent to over 100 gm. of common salt per liter of leaf-tissue fluids.

The greasewood-shadscale (*Sarcobatus vermiculatus* and *Atriplex confertifolia*) association occupies a belt lying between the shadscale association and the grass flats and salt flats. It also occupies the ridges and hummocks which diversify the highly saline mud flats along the southern shore of the Great Salt Lake. Soil moisture and salinity are much higher than in any of the preceding associations, and agricultural development must involve irrigation, and in some places drainage as well. The average osmotic concentration of the leaf sap of the woody species is over 30 atmospheres, the average specific electrical conductivity is over 0.045 mho, and the average chlorid content over 18 gm. of Cl per liter.

The grass flats occupy land of high moisture content but of highly variable salinity. The physicochemical constants of the tissue fluids are closely correlated with the salinity of the ground water. The values of osmotic concentration, specific electrical conductivity, and chlorid content are, roughly speaking, comparable with those found in the preceding and the following associations. Except when the conditions can be modified by drainage (which is generally impracticable because of the low level of the land), the grass flats have greater value for grazing or for natural hay production than for any other crop.

The salt flats are characterized by soil of exceedingly fine texture which is moist for a large part of the year. In late summer salt crystallizes out over the surface. Plants are found only on small hummocks or along drainage channels from springs. *Allenrolfea occidentalis* and *Salicornia utahensis*, both stem succulents, are the chief species. These are both characterized by an osmotic concentration of about 40 atmospheres, a specific electrical conductivity of over 0.060 mho, and a chlorid content of 17 to 30 gm. per liter of tissue fluid.

From the foregoing outline of the findings as to matters of fact, it is clear that there is a close parallelism between the physicochemical properties of the tissue fluids of the native species on the one hand and the characteristics of the soil and the capacity of the land for crop production on the other.

The fact that few agricultural plants of importance have sap properties similar to those of the native desert species probably furnishes the explanation of the failure of crop plants in the more severe of the habitats of this region except as their conditions are modified by irrigation.

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# INVESTIGATIONS ON THE NEMATODE DISEASE OF CEREALS CAUSED BY *TYLENCHUS TRITICI*<sup>1</sup>

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## INTRODUCTION

The nematode disease of wheat, caused by *Tylenchus tritici* (Stein.) Bast. (25, 3)<sup>3</sup>, has been known to be prevalent for many years in Europe, where at times it has caused severe losses. The discovery in 1918 that it was destructive in certain parts of the United States led the Office of Cereal Investigations of the Bureau of Plant Industry to begin a study of the trouble with a view to controlling or completely eradicating the disease. The history of the disease and a description of the causal organism, together with certain phases of its physiology, were given by Byars (6) in 1920. The purpose of the present publication is to present the results of the more recent studies of the disease, including a detailed description of the common symptoms.

## COMMON NAME

The nematode disease of cereals, caused by *Tylenchus tritici*, has been given various names in the countries where it has been observed. In Germany, owing to the similarity of the galls<sup>4</sup> to seeds of cockle, it is called "Radekrankheit" (cockle disease), and on account of the gnarled appearance of infected plants, it is also called "Gichtkrankheit" (gout disease). In France it was confused for a long time with bunt or stinking smut and therefore referred to as "blé nielle." In England, according to Ormerod (19), the disease has been called "purples," owing to the purple tint of the galls at a certain stage of their development. It is known there also as cockles, ear cockle, and false ergot. In the United States the disease is known by various names, among which the most common are eelworm or nematode disease, cockle wheat, hard smut, and nematode gall. The name nematode disease is used in this publication.

## GEOGRAPHICAL DISTRIBUTION

The nematode disease of wheat is easily spread long distances by means of the galls mixed in the seed wheat. As a result, its distribution is almost world-wide. Sorauer (24, p. 26-30) states that it occurs in France,

<sup>1</sup> Received for publication March 11, 1924. The major portion of the investigations herein reported was conducted at the Arlington Experiment Farm, Rosslyn, Va. The rotation studies were conducted on the farms of W. S. Finnell, Morrisville, Va., and J. E. Riffe, Woodstock, Va., whose generous cooperation together with that of B. A. Warriner, county agent, Woodstock, Va., assisted greatly in making this phase, of the investigations possible. A considerable portion of the research was conducted at Madison, Wis., in cooperation with the Wisconsin Agricultural Experiment Station.

<sup>2</sup> The writer wishes especially to express his appreciation for the assistance in various ways of Dr. L. P. Byars, formerly Pathologist, Office of Cotton, Truck and Forage Crop Disease Investigations, through the early stages of the investigation, and to Dr. A. G. Johnson for the many helpful suggestions and other valuable assistance rendered throughout the progress of the studies and in the preparation of the manuscript.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," pp. 954-955.

<sup>4</sup> Throughout this paper the word galls will be used to designate the common nematode galls found in wheat infected by *Tylenchus tritici*.

Germany, Austria, Hungary, Switzerland, Italy, Sweden, Holland, England, and Australia. Byars (6) found galls in wheat from Russia, India, China, and Turkestan. Averna-Sacca (2, *p.* 234) observed the disease in Brazil in 1912. Among the British African colonies and protectorates, Dr. E. J. Butler states, in correspondence, that it has been reported only from Egypt. It has not been reported from Central America, Mexico, or Canada.

According to Johnson (15), it was first reported in the United States in 1909 in California, West Virginia, New York, and Georgia. Later it was found widely distributed in Virginia, which State has since been found to contain the chief area of infestation. At present there are no indications of the disease in New York or California. Recent surveys have revealed its presence in 53 counties in Virginia, 11 in West Virginia, 2 in North Carolina, 1 in South Carolina, and 1 in Georgia. Probably a wide and intensive survey would show a general spread of the trouble outward from the badly infested region in the western part of Virginia. .

The disease usually is found by the discovery of galls in the grain and screenings at the mills. However, it sometimes is difficult to determine the source of the gall-infested grain found in a mill. For example, galls found in a Maryland mill were later traced to wheat shipped in from Virginia. Gall-infested wheat in a mill in Ohio was found to have come from West Virginia. The report of the disease in New York likewise may have been the result of galls in wheat shipped in from an infested area in another State.

#### ECONOMIC IMPORTANCE

Some of the earlier European workers in Germany, Austria, and England mention the nematode disease as the cause of severe losses. Doubtless these losses were due largely to ignorance concerning the nature of the disease and the methods for its control. At present its economic status in Europe is of minor importance, probably on account of improved farm practice, such as rotation of crops and the use of clean seed.

During the recent war the disease became very severe in certain sections of the southeastern United States, owing to the lack of rotation incident to the intensive wheat-production program. It was found that wheat or rye grown on land infested by a nematode-diseased crop the previous year usually became severely infected and was greatly reduced in yield. In wheat the losses were sometimes as high as 50 per cent, and according to Byars (5) losses as high as 70 per cent have been reported. In certain cases rye was even more severely injured than wheat, one field in Virginia in 1919 being a total loss. In addition to the reduction in yield, according to Coleman and Regan (8), the quality and consequently the market value also are greatly reduced, owing to the presence of the galls in the grain.

In order to determine the effect of the disease on rye, the following experiment was conducted in 1920. Seed of winter rye was mixed with an equal volume of galls from wheat and sown in plats on the Arlington Experiment Farm. At maturity the plants from both the inoculated and uninoculated plats were pulled. Representative infected plants were selected from the inoculated plat and a similar lot of healthy plants was taken from the uninoculated plat. From the infected plants four lots of 100 culms each were cut off immediately above the crown. From



the healthy plants three lots of 100 culms each were similarly cut. In no case was any special selection made, the culms being taken as they came. Each lot of 100 culms from the infected plants was hand-threshed separately. The galls were separated from the kernels in each case, and for each lot the weights of the straw, kernels, and galls were determined separately. Similarly, each lot of 100 culms from the healthy plants was hand-threshed separately. In each case the weights of the grain and of the straw from each lot were secured. The results are given in Table I.

TABLE I.—Weight of straw and weight and volume of threshed grain from three lots of healthy and four lots of nematode-infected rye culms, 100 culms in each lot, Arlington Experiment Farm, Rosslyn, Va., June, 1920

Lot No.	Yield of straw from—			Yield of grain from—						Volume of grain from—				
	Healthy culms.		Infected culms.	Healthy culms.	Infected culms.*				Healthy culms.	Infected culms.				
					Kernels.		Galls.			Kernels.		Galls.		
					Weight.	Reduction.	Weight.			Volume.	Reduction.	Volume.		
	Gm.	Gm.	P. ct.	Gm.	Gm.	P. ct.	Gm.	P. ct.	Cc.	Cc.	P. ct.	Cc.	P. ct.	
1 .....	311.9	178.6	43	141.7	49.0	65	4.5	8	190	70	63	15	18	
2 .....	269.3	174.1	35	113.4	38.5	66	6.0	13	150	55	63	15	21	
3 .....	212.6	151.2	29	85.0	33.0	61	5.5	14	110	40	64	10	20	
4 .....		157.2			27.0		4.5	14		45		20	31	
Average....	264.6	165.3	36	113.4	36.9	64	5.1	12	150	52.5	63	15	22	

It is of especial interest to note that although the average percentages of galls are only 12 and 22 per cent by weight and by volume, respectively, the actual average reduction in the yield of kernels from infected plants as compared with the yield from uninfected plants is 64 and 63 per cent by weight and by volume, respectively. This seems to indicate that the percentage by weight or volume of galls in infested grain does not truly represent the actual reduction in yield caused by the disease. This is shown also in Tables VII and VIII.

Reports of reduced yields caused by the nematode disease may vary widely owing to the different methods used to determine the amount of reduction. Percentages of infection may be based on the relative number of seedlings showing symptoms, the relative number of heads containing galls, or the percentage of galls in the threshed grain. Head counts may take into consideration partially infected and totally infected heads. The percentage of galls in the threshed grain may be based on the relative number, volume, or weight of galls and kernels. In order to compare these different methods of taking and recording percentages of infection, the data were tabulated from an experiment conducted at the Arlington Experiment Farm in the crop year 1919-20, in which the relative susceptibility of wheat, rye, emmer, and spelt was studied. These percentages are given in Table II.

TABLE II.—Percentage of infected seedlings and infected heads and percentage of galls, by number, volume, and weight, in the threshed grain of different varieties of winter wheat, winter rye, winter emmer, and winter spelt grown in inoculated soil at the Arlington Experiment Farm, Rosslyn, Va., during the crop year 1919-20

Variety.	C. I. No.	Percentage of infection based on—				
		Percentage of infected—		Percentage of galls by—		
		Seedlings.	Heads.	Number.	Volume.	Weight.
Winter wheat:						
"Arkansas Red Wonder".....		67	74	51	33	23
Bearded Purple Straw.....	1911	73	88	83	69	59
Bearded Winter Fife.....	1942	66	80	52	31	22
Brown Fife.....	1933	68	63	43	28	14
China.....	180	62	80	61	42	31
Currell.....	3326	72	80	63	48	30
Dietz Longberry.....	1981	52	64	40	26	16
Genesee Giant.....	1744	66	85	61	41	29
Illini Chief.....	5406	62	66	47	29	18
Kanred.....	5146	2	T.			
Leap.....	4832	50	95	54	35	24
Mediterranean.....	3115-2	57	87	65	46	33
Do.....	1912-15	68	93	73	46	41
Nebraska No. 28.....	5147	37	44	52	25	11
New Amber Longberry.....	1973	63	93	68	51	36
Poole.....	3489	65	77	50	31	20
Potomac.....	1733	71	65	51	31	20
Purplestraw.....	1915	65	91	70	52	40
Red Fultz.....	3413	45	71	58	36	24
Shepherd.....	6163	52	93	72	56	42
Stoner.....	2980	73	88	73	55	42
Average.....		59	75	57	39	27
Rye:						
Abruzzes.....	40	26	96	40	12	5
Rosen.....	195	35	94	53	19	10
Von Rümker No. 2.....	174	31	98	55	21	11
Winter.....	208	56	65	56	22	11
Average.....		37	88	51	19	9
Emmer:						
Black Winter.....	2337	25		25		
White Winter.....	3628	71		27		
Average.....		48		26		
Spelt:						
Alstroum.....	1773	28		6		
Bearded Winter.....	1724	40				
Red Winter.....	1772	35				
Average.....		34				

From a comparison of these methods, as shown in Table II and graphically in Figure 1, it seems that the percentage of infection based on head counts invariably is the highest, while that based on the relative weight of galls and kernels is the lowest. However, in the case of winter wheat, none of these methods takes into consideration the reduction in stand following the use of nematode-infested seed.

During the crop season 1921-22, the following experiment was conducted at Madison, Wis., to determine if reduction in stand followed the use of nematode-infested seed: Turkey wheat was sown in four plats of equal size. The first plat was sown with uninfested seed. The seed sown in the second plat was mixed with 5 per cent of its weight in galls. In the third plat the seed was mixed with 15 per cent and in the fourth

plat with 45 per cent of its weight in galls. The resulting stands and yields are given in Table III.

TABLE III.—Number of plants and culms and weight of grain from Turkey wheat unin-fested with galls and infested at seeding time with different quantities of galls, together with reduction in stand and yield and percentage by count of galls in threshed grain from inoculated plats at Madison, Wis., in 1922

Percentage by weight of galls sown in seed.	Plants.		Culms.		Grain.		Percent-age by count of galls in grain.
	Number.	Percent-age of reduction.	Number.	Percent-age of reduction.	Weight (in grams).	Percent-age of reduction.	
0.....	1,215	.....	3,330	.....	935	.....	0
5.....	1,105	9	2,530	24	695	26	7
15.....	400	67	1,375	59	425	55	20
45.....	310	74	940	72	220	76	30

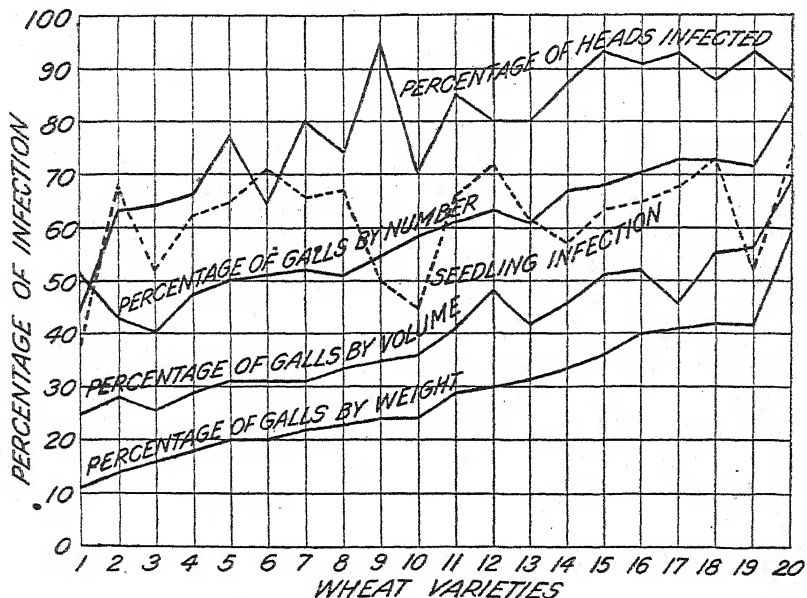


FIG. 1.—Graph comparing the results of determining by different methods the percentages of nematode disease infection in different varieties of wheat as shown in Table II.

There seemed to be a consistent relation between the quantity of infecting material used and the resulting reduction in the stand. Fromme (10) found a similar relation between the percentage of galls in the seed and the resulting severity of infection.

Estimates of reduced yield due to nematode injury, based on the number of seedlings showing symptoms of such injury, are inaccurate, because it was found that many plants showing such symptoms as seedlings did not always produce galls at maturity. Eighty plants showing typical symptoms of nematode injury were tagged as seedlings at the Arlington Experiment Farm in April, 1919, and at maturity 15 of them,

or 18.75 per cent, produced gall-free heads. On the other hand, numerous seedlings with no apparent symptoms whatever upon dissection and microscopic examination proved to harbor an abundance of nematodes. In October, 1919, a number of varieties of wheat, rye, emmer, and spelt were sown with a heavy inoculation of galls in rod rows at the Arlington Experiment Farm. In the spring the percentage by count of seedlings showing signs of nematode injury was determined. At maturity the percentage of infected heads in each variety of wheat and rye was determined. Then the plants of each of these varieties were separately threshed in a head thresher. In the threshed grain of each variety the percentages of infection based on the number, volume, and weight, respectively, of galls were determined. The results as set forth in Table II indicate that some varieties showing pronounced symptoms as seedlings did not produce as many or as badly diseased heads as other varieties in which seedling injury was less evident.

Twelve heads of rye were carefully threshed by hand and the galls and kernels in each head counted, as shown in Table IV. An examination of these and numerous other diseased heads showed that the degree of infection in the spikes varied all the way from heads producing all galls and no kernels to those having only one or two galls and 30 or more kernels.

TABLE IV.—Number of galls and kernels in infected heads of rye at the Arlington Experiment Farm, Rosslyn, Va., in 1920

Head No.	Galls.	Kernels.	Head No.	Galls.	Kernels.
1.....	4	10	7.....	67	1
2.....	27	15	8.....	54	11
3.....	23	3	9.....	12	13
4.....	12	35	10.....	39	20
5.....	44	10	11.....	11	37
6.....	30	20	12.....	1	26

An estimate of reduced yield based on the relative number of infected heads apparently is even more inaccurate than one based on the relative number of seedlings showing symptoms of nematode injury. Furthermore, this method of determining reduction in yield does not take into consideration the plants killed as seedlings and those prevented from heading.

Estimates of yield reduction based on the relative number, volume, or weight of galls and kernels in the infested grain after threshing are likely to be too low for the following three reasons:

Many infected plants are killed as seedlings. During April, 1919, 500 diseased seedlings in the plats at the Arlington Experiment Farm were labeled. During the latter part of May they were again examined and 70 of them, or over 14 per cent, were found dead.

Many of the diseased plants, although producing only a few galls, are so badly stunted that at maturity they also produce very few kernels. Thus, although the relative number of galls is not greatly increased, the actual yield of grain is more than proportionately reduced.

The threshed grain contains relatively fewer galls than would be found in a hand-threshed sample, as the galls are relatively light in weight and many are blown out with the straw and screenings at threshing time.

Even in a hand-threshed sample, the percentage by number of galls in the grain is not a trustworthy basis for estimating reductions in yield,

for as many as five galls may replace a single kernel. Likewise, the percentage by weight is inadequate, as the galls are very light in comparison with the kernels.

The relative *volume* of galls and kernels, while far from being an ideal or accurate basis for estimating reductions in yield, seems to be the most nearly adequate. In order to determine accurately the reduction in yield, it is necessary to compare the yields of infected and uninfected crops grown under parallel conditions. Limited data of this kind were obtained for rye in connection with another experiment. These results are given in Tables VII and VIII.

Thus far the nematode disease of cereals has not been reported from any part of the spring-wheat region. Experiments were conducted at the Arlington Experiment Farm in 1920 to determine how this disease would affect spring wheat. In March 3-rod rows of each of 13 varieties of spring wheat were sown in soil which had been heavily inoculated with galls during the previous October and also in soil to which galls were applied at the time of seeding. Two rows of each variety were sown on clean soil at the same time. No infection occurred in the uninoculated control rows, but unfortunately these plants were destroyed by sparrows before harvest to such an extent that reliable yield data could not be secured from them.

The percentage of seedling injury in the inoculated rows was determined by noting the number of diseased plants per 100 in three representative parts of each row. At maturity the plants from each set of rows were threshed and the percentages of galls in the grain by number, volume, and weight were determined. The results are given in Table V.

TABLE V.—Percentages of diseased seedlings and percentages of galls by number, volume, and weight in the threshed grain of different varieties of spring wheat grown on soil inoculated with galls in the fall and on soil inoculated at time of sowing in the spring at the Arlington Experiment Farm in 1920

Variety.	C. I. No.	Fall-inoculated soil.				Spring-inoculated soil.			
		Seed- ling infection.	Galls in threshed grain.			Seed- ling infection.	Galls in threshed grain.		
			Num- ber.	Vol- ume.	Weight.		Num- ber.	Vol- ume.	Weight.
		<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>	<i>Per cent.</i>
Arnautka.....	4064	32	30	13	7	46	63	40	27
Baart.....	1697	50	41	21	13	85	97	93	90
Dicklow.....	3663	60	36	16	13	85	96	92	95
Hard Federation.....	4733	80	24	11	5	96	99	99	99
Haynes Bluestem.....	2874	30	9	3	3	70	95	88	83
Kota.....	5878	32	30	14	7	80	87	71	63
Kubanka.....	1440	24	37	16	9	70	88	74	71
Marquis.....	3641	33	8	4	2	70	81	60	48
Monad.....	3320	16	69	44	32	70	82	64	55
Pentad (D-5).....	3322	37	63	66	26	68	81	62	50
Power.....	3697	56	37	19	14	83	95	92	91
Prelude.....	4323	90	80	61	40	98	99	96	94
Ruby.....	6047	60	32	20	11	95	99	88	82
Average.....		46	38	24	14	78	89	78	73

It will be noted that unusually severe infections were obtained in all 13 varieties on the spring-inoculated soil. On the soil inoculated in the fall a number of the varieties showed considerable infection. In this latter series it is of especial interest to note that two varieties, Haynes Bluestem and Marquis, showed a moderately high degree of seedling injury but low percentages of infection in the threshed grain. Such variations in these or other varieties were not evident on the soil inoculated at sowing time in spring. This may have been because of the unusually severe infection, as practically all the plants grown on the soil inoculated at the time of sowing were badly stunted and deformed and produced but few normal kernels (Pl. 1, E). Infection was much less severe on the soil inoculated in the fall, some of the varieties even comparing favorably in vigor, and apparently in yield, with those grown in the uninoculated control rows.

A similar experiment with fewer varieties was conducted at Madison, Wis., in 1921, with similar results. In one plat the galls were placed in the soil on October 7, 1920, and in another plat on April 7 the following spring, at which time four varieties of spring wheat were sown in both plats and also in an adjacent uninoculated control plat. No infections occurred in the latter. The data on seedling injury in the inoculated plat were obtained as before by observing 300 plants in each plat. The amount of infection is shown in Table VI.

TABLE VI.—Percentage of plants showing seedling injury and percentages by volume of galls in the threshed grain from four varieties of spring wheat grown on soil inoculated with galls in the fall and on soil inoculated at time of sowing at Madison, Wis., in 1921

Variety.	Seedling injury on—		Percentage by volume of galls in threshed grain.	
	Soil inoculated in the fall.	Soil inoculated in the spring.	Soil inoculated in the fall.	Soil inoculated in the spring.
	<i>Per cent.</i>	<i>Per cent.</i>		
Marquis.....	5	66	Trace.	71
Prelude.....	10	72	10	95
Huron.....	2	80	6	80
Unnamed hybrid.....	6	84	4	70
Average.....	6	76	5	79

It seems evident that although the nematodes can overwinter to some extent in the soil in Wisconsin, the only important source of infection of spring wheat would be infested seed. The use of such seed results in an almost worthless crop. This experiment with spring-wheat varieties was repeated in 1922 at Madison with similar results. The stand and yield of the wheat grown on the soil inoculated in the spring were practically negligible, while those of the wheat sown in soil inoculated the previous fall were quite good.

Rye was included in the latter experiment and, although the soil inoculated in the spring did not cause as severe an infection as in the case of the wheat, yet the yield was considerably reduced. The rye grown on fall-inoculated soil was not appreciably injured. The results are given in Table VII.

TABLE VII.—Comparative seedling injury and infection percentages and yields of Wisconsin spring rye grown on soil uninoculated and inoculated with galls in the fall and in the spring, respectively, at Madison, Wis., in 1922

Plot.	Seedling injury.	Volume of yield.	Reduction in volume.	Weight of yield.	Reduction in weight.	Volume.		
						Kernels.	Galls.	Galls.
	Per cent.	Cc.	Per cent.	Grams.	Per cent.	Cc.	Cc.	Per cent.
Control uninoculated.	0	350	0	262	0	350	0	0
Fall inoculated.....	2	350	0	253	3	348	2	Trace.
Spring inoculated....	16	250	29	172	34	220	30	12

Winter wheat and rye also were sown at Madison in the fall of 1920, and the soil was inoculated with galls at the time of sowing. The results were very similar to those obtained in the southeastern United States. The symptoms of the disease appeared in the fall, the organisms were found between the leaf sheaths of the plants throughout the winter, and at harvest time an abundance of galls was found in the heads. On account of injury caused by sparrows, complete data on the wheat were not obtained. The rye, however, was not injured by the birds and produced the results shown in Table VIII.

TABLE VIII.—Percentage of seedling injury, volume and weight of grain from inoculated and uninoculated plots of Wisconsin winter rye, volume and percentage by volume of galls, and percentage reduction in yield in the threshed grain from the inoculated plots at Madison, Wis., in 1922

Row.	Seedling injury.	Volume of total yield.	Reduction.	Weight of total yield.	Reduction.	Volume.		
						Kernels.	Galls.	Galls.
	Per cent.	Cc.	Per cent.	Grams.	Per cent.	Cc.	Cc.	Per cent.
Uninoculated control.	.....	560	.....	407	.....	560	0	.....
Inoculated.....	60	325	42	215	47	275	30	10
Inoculated.....	60	320	43	202	50	250	40	14

From these experiments we may conclude that the nematode disease could cause serious losses in the spring-wheat region if, unfortunately, it were introduced there. Gall-infested seed when sown in spring insures the presence of enormous numbers of active larvae which are not subjected in the free-living state to the unfavorable conditions obtaining in the winter and thus are able to bring about severe infection.

#### HOSTS

Considerable disagreement exists among investigators as to the host range of the wheat nematode, although all have secured abundant infection in wheat.

Roffredi (21, 22) states that he obtained infection in rye, barley, and spelt.

Marcinowski (17, p. 67-117) tried to infect rye, spelt, barley, and oats. Galls were found in the first two only, although larvae in abundance were found between the leaf sheaths of all four. She states that the rye galls were small and that the larvae in them were not so far developed as those found in the galls in wheat. Spelt did not produce as many

galls in the heads as were used in inoculating the seed. Only one barley plant was found showing symptoms of nematode injury in the seedling stage, while oats seemed to be entirely immune. Rye, barley, and oats, Marcinowski concludes, are not to be considered congenial hosts for the wheat nematode. Henslow (12) claims to have secured slight infection in oats, barley, and rye.

In experiments extending over two years, Byars, Johnson, and Leukel (7) found rye to be fully as susceptible to the nematode disease as wheat and in some cases even more severely affected. The resulting galls usually are smaller than those in wheat and also lighter in color (Pl. 5, H), but, contrary to Marcinowski's findings, the larvae are as fully developed in the rye galls as are those found in wheat galls. Galls obtained from rye heads were used for inoculating both wheat and rye, and abundant infection was obtained in both cases. The infection percentages obtained by inoculating four varieties of rye with nematode galls at the time of seeding are given in Table II.

Two varieties of emmer (*Triticum dicoccum* Schr.), Black Winter and White Winter, also were found to be extremely susceptible and were characterized by an unusually large number of leaf galls (Pl. 1, B and C). The number of galls obtained upon threshing constituted over 25 per cent of the grain.

Spelt (*Triticum spelta* L.), while more resistant than most of the varieties of wheat, rye, and emmer used in the experiment, nevertheless is to be classed as a susceptible host. It showed abundant symptoms of nematode injury in the seedling stage, and 6 per cent of the volume of the hand-threshed grain consisted of galls. The varieties grown were Alstroum, Bearded Winter, and Red Winter.

The following varieties of oats were sown at the Arlington Experiment Farm in 1920 and galls sown with the seed: Aurora, Culberson, Fulghum, Red Rustproof, and Winter Turf. Red Rustproof was found to be slightly injured in the seedling stage (Pl. 2, D and E), and in a few cases small galls formed while the heads were still in the boot, but up to the present time no galls have been found in the ripened grain. In order to determine whether the yield of spring oats can be reduced appreciably by the wheat nematode (*Tylenchus tritici*), seed of two varieties was sown with an equal volume of galls at Madison, Wis., in April, 1922. An equal quantity of uninoculated seed was used as a control. Observations were made at intervals during the growing period, and at maturity each row was threshed separately and the yield recorded (Table IX). In no case was there any perceptible difference in stand, vigor, or appearance between the inoculated oats and the control. Sparrow injury made exact yield data impossible, but such results as were obtained seemed to indicate that nematode injury did not affect the yield.

The following five varieties of barley were used in infection experiments at the Arlington Experiment Farm (1919-1920): Hansee Hull-less, Squarehead Winter, Tennessee Winter, Texas Winter, and Wisconsin Winter. Hansee Hull-less was the only one which showed any symptoms of nematode injury (Pl. 3, A and B) although the others often were found to contain larvae between the leaf sheaths. Of 186 ripe heads of Hansee Hull-less barley, only 3 contained galls (Pl. 3, B). These were small and shriveled, but contained larvae similar to those in galls from wheat. Wheat and barley, similarly inoculated, are shown in



Plate 4. It will be noted that, while the wheat is badly stunted, the barley apparently is uninjured. On the assumption that hull-less barley might be more susceptible than hulled barley, seeds of several hull-less varieties were sown at Madison, Wis., (1922) with a heavy inoculation of galls but in no case was infection obtained. As in the case of oats, comparative data were taken on the inoculated barley and on uninoculated controls, and no differences were observed in comparative vigor, stand, or yield (Table IX).

TABLE IX.—Yields of five 10-foot rows of barley and oat varieties, three inoculated and two uninoculated with galls of the wheat nematode at the time of sowing, at Madison, Wis., in 1922

Variety.	Inoculated.				Uninoculated.		
	Row 1.	Row 2.	Row 3.	Average.	Row 1.	Row 2.	Average.
Barley:	<i>Grams.</i>	<i>Grams.</i>	<i>Grams.</i>		<i>Grams.</i>	<i>Grams.</i>	
White Hull-less.....	110	105	70	95	82	85	84
Nepal.....	83	80	86	83	86	82	84
Himalaya.....	141	155	148	148	140	144	142
Black Hull-less.....	270	311	293	291	254	252	253
Oats:							
Golden Sheaf <sup>a</sup> .....	220	100	155	158	272	226	249
Wisconsin Pedigree No. 7 <sup>a</sup> ..	190	300	250	247	270	460	365

<sup>a</sup> Injury by sparrows makes results inconclusive.

Grasses appear to be immune from the wheat nematode. In experiments extending over two years, Marcinowski (17, p. 67-117), using nematodes from wheat, tried to infect 15 species of grasses belonging to seven genera. Although she used an abundance of inoculating material, several hundred galls per plant, she secured no infection. In many plants dissected and examined, numerous larvae had gathered about the growing point, but all the remaining plants matured normally and produced seed without any indication of gall formation.

Similar experiments conducted by the writer at Madison, Wis. (1921-22), also gave negative results. Seeds of several species of *Agrostis*, *Arrhenatherum*, *Festuca*, *Lolium*, *Phleum*, and *Poa* were inoculated at time of sowing with both whole and ground galls. The inoculated plants at no time differed in any respect from the uninoculated control. Larvae in small numbers sometimes were found about the growing points of dissected plants, but no disease symptoms were evident and the plants matured normally.

Neither do the nematodes producing galls on certain grasses seem able to infect wheat. Bessey (4) tried to infect wheat by sowing it with nematode galls from the following seven genera of grasses: *Agropyron*, *Agrostis*, *Calamagrostis*, *Chaetochloa*, *Elymus*, *Sporobolus*, and *Trisetum*. He secured no infection whatsoever. The writer inoculated wheat at seeding time with both galls and free larvae from *Calamagrostis*, but in no case were any symptoms of nematode injury observed or head infection secured.

*Tylenchus tritici* is a highly specialized parasite requiring either wheat or a nearly related host in order to multiply rapidly. Therefore, land infested by the wheat nematode may be sown to grass or even to oats or barley without danger of perpetuating or spreading the disease.

## DESCRIPTION

## VEGETATIVE SYMPTOMS

The first evidence of nematode injury in the seedling plant is the appearance of slight elevations on the upper side of the leaf with corresponding indentations on the lower side. These elevations soon become more pronounced and the leaf sometimes takes on a somewhat yellowish, mottled appearance (Pl. 1, D). The leaf may become wrinkled, beginning usually at the edge of the blade. This wrinkling, however, often is found to a slight extent on healthy plants. As this condition becomes aggravated, the leaf margins curl in from the edges toward the midrib on the upper side very much as in some cases of aphid injury, and frequently the leaf is split in one or more places. This rolling or curling leaf often incloses the emerging blade of a younger leaf, thus retarding its normal growth and causing it to become more or less distorted and "buckled" (Pl. 2, A). As this younger leaf is not free to grow longitudinally, it often grows in a zigzag manner, frequently while within the inclosing older leaf sheath, causing the latter to assume a swelled or bulged appearance. This hindrance to the growth of the leaf tip often causes another deformity, namely, the bending of the culm, as shown in Plate 2, C. A similar restriction to the growth of several successive leaves may bring about an alternate curving of the stem in opposite directions.

The slight clockwise twist occurring naturally in wheat leaves often is so accentuated in the diseased plants that the leaves form a rather tight spiral roll, as shown in Plate 2, B. Sometimes only a part and sometimes the whole of the leaf is involved. When the plant approaches the heading stage, the flag leaf often becomes wound tightly about the stem, thus interfering considerably with the emergence of the head from the boot (Pl. 1, A). The awns frequently are somewhat distorted when they issue from the inclosing sheath, but they often assume their normal appearance later.

As the plant approaches maturity, some of the earlier symptoms may disappear. This was proved by tagging a large number of infested seedlings in April, 1919, and observing them at intervals until maturity. Some of the badly distorted leaves were found withered and dead, while those formed later showed no pronounced symptoms. This, however, is not of general occurrence, especially if the plant is badly deformed in the seedling stage, for in the majority of the plants observed symptoms on the vegetative parts were evident at maturity. Of the plants examined on April 20, 1919, 62 per cent showed symptoms. On May 22 another count was made and 58 per cent still showed symptoms.

It may be well to add that similar symptoms often are caused by other agencies, such as aphids, the Hessian fly, drought, and some fungous diseases. Neither does the absence of symptoms indicate freedom from the disease, for often a plant which appears healthy in every respect produces an infected head. Wheat with symptoms similar to nematode injury, but which probably are due to drought, is shown in Plate 2, F.

## HEAD SYMPTOMS

The diseased heads generally differ from the healthy heads in size and shape, and sometimes in color. They also remain green longer than healthy ones. A healthy wheat head between two diseased heads is shown in Plate 3, C and D. Occasionally, however, we find diseased heads which compare favorably in size with uninfected ones. Especially is this true when they contain but few galls. The glumes of the diseased ears stand out more nearly horizontally, especially when immature, owing to the greater diameter of the galls as compared with that of the kernels. This makes the heads resemble somewhat those infected with bunt. In rye this is not so much the case, for the galls in this cereal usually are smaller than the rye kernels, and, unless a head is badly diseased, it may closely resemble a healthy one (Pl. 3, E and F).

The infected culms, as a rule, are shorter than the healthy ones. Appl (1) gives an average difference between the two of about 10 cm. A similar shortening was observed in the infected wheat and rye in the experimental plats at the Arlington Experiment Farm. Measurements of several hundred healthy and diseased culms of wheat showed an average difference of 12 cm. Data on the weight of the straw and grain from normal and diseased rye plants showed that the reduction in the yield of straw and grain from infected plants was considerable (Table V).

If one head in a stool is infected it does not necessarily follow that the others in that stool also are diseased. Just as we may have galls and normal kernels in the same head, so also there may be both diseased and healthy heads in the same plants. This fact was established by sowing heavily inoculated kernels several inches apart so that the plants would stool profusely. The plants were examined carefully at maturity and the individual heads threshed. The data obtained are presented in Table X.

TABLE X.—Number of healthy and infected heads in individual stools of wheat grown on inoculated soil at the Arlington Experiment Farm, Rosslyn, Va., in 1920

Number of culms in the stool.	Infected heads.	Healthy heads.	Shattered <sup>a</sup> and sterile heads.
6.....	5	1	0
4.....	3	1	0
4.....	2	2	0
12.....	8	0	4
4.....	1	3	0
15.....	4	9	2
9.....	4	3	2
6.....	3	3	0
9.....	0	1	2
16.....	1	7	8
8.....	5	2	1
13.....	0	2	5

<sup>a</sup> Shattered heads include those partly or wholly threshed by birds or by handling before final data were obtained.

## NUMBER AND ARRANGEMENT OF GALLS IN SPIKELETS

In healthy wheat and rye a spikelet usually produces two or three kernels, one in each developed floret. But in wheat affected with the nematode disease a floret often produces two to four galls, formed from

the different organs, and four, five, or even more gall-producing florets may develop within a spikelet. In 25 diseased heads of wheat, there were found 2 spikelets with only 1 developed floret each, 85 spikelets with 2 florets, 120 with 3, 87 with 4, 30 with 5, and 4 with 6. In these same spikelets, 33 contained only 1 gall each, 108 contained 2 galls each, 98 contained 3 galls, 70 contained 4 galls, 28 contained 5 galls, 6 contained 6 galls, 2 contained 7 galls, and 1 contained 9 galls. Some of these data are presented in Table XI.

TABLE XI.—*Number of florets per spikelet and the contents of the florets in each spikelet in nematode-infected wheat heads from plants grown at the Arlington Experiment Farm, Va., in 1920*

Spikelet No.	Florets.	Galls.	Kernels.	Spikelet No.	Florets.	Galls.	Kernels.
1.....	2	3	0	37.....	3	2	1
2.....	4	4	1	38.....	3	2	1
3.....	4	3	2	39.....	4	3	1
4.....	5	2	3	40.....	3	3	1
5.....	3	3	1	41.....	3	2	1
6.....	5	4	1	42.....	4	3	1
7.....	3	4	0	43.....	4	5	1
8.....	6	6	1	44.....	3	2	1
9.....	4	5	0	45.....	3	2	1
10.....	5	6	0	46.....	2	0	2
11.....	4	3	1	47.....	4	3	1
12.....	4	5	0	48.....	3	2	1
13.....	4	5	0	49.....	4	4	0
14.....	2	3	0	50.....	1	1	0
15.....	3	1	2	51.....	5	4	1
16.....	3	2	1	52.....	2	1	1
17.....	4	3	1	53.....	3	2	1
18.....	3	2	1	54.....	2	0	2
19.....	4	2	2	55.....	4	3	1
20.....	3	2	1	56.....	3	2	1
21.....	3	2	1	57.....	4	3	1
22.....	5	5	0	58.....	3	2	1
23.....	6	9	0	59.....	3	1	2
24.....	4	5	0	60.....	3	2	1
25.....	4	5	0	61.....	3	2	1
26.....	5	6	0	62.....	1	0	1
27.....	6	7	0	63.....	2	2	0
28.....	5	7	0	64.....	2	1	1
29.....	4	3	1	65.....	3	2	1
30.....	5	4	1	66.....	3	2	1
31.....	5	4	1	67.....	4	3	1
32.....	4	4	1	68.....	4	3	1
33.....	4	3	1	69.....	3	4	0
34.....	4	5	0	70.....	4	4	0
35.....	4	4	0	71.....	5	5	0
36.....	4	3	1	72.....	4	6	0
Average.....					3.6	3.2	0.8

#### DESCRIPTION OF GALLS

In the earlier stages of their formation the young galls can be distinguished from the naturally forming kernels by their greener color, their glistening smoothness, and the absence of the feathery pistil. As the heads approach maturity the galls are readily distinguished from the kernels by their shape, greener color, and the manner in which they cause the glumes to spread, due to their greater diameter.

The mature galls in winter wheat, emmer, and spelt are alike in size and appearance, but considerable difference was observed in the shape and color of the galls in some spring wheats, especially Prelude.

The galls in wheat are from 3.5 to 4.5 mm. in length and from 2 to 3 mm. in width. Variations from these dimensions are frequent, some galls being larger than wheat kernels and others very much smaller.

They usually are dark, almost black, in color, but the proximal ends invariably show a light-brown coloration. The distal ends often have one or more characteristic beaklike projections. The galls, like the wheat kernels, generally are furrowed on one side, but they usually are shorter and proportionately thicker (Pl. 5, G). They are without pubescence of any kind.

Galls in rye, as a rule, are longer in proportion to their width than those in wheat and are more irregular in form. In length they vary from 2 to 4.5 and even 5 mm., and in width from 1 to 2.5 mm. They are decidedly lighter in color than galls in wheat, being of a buff shade, much like rye kernels. The difference between galls in rye and wheat and the variation in the size of galls in rye are shown in Plate 5, G and H.

In addition to the galls formed in the flower, galls sometimes are formed on the leaves (Pl. 1, B and C). How these galls originate has not been determined, but they probably begin to form before the leaf emerges from the sheath. They bear no resemblance to flower galls in appearance, being very much wrinkled and irregular in shape. They contain mature nematodes when still green and at a later stage are full of larvae. As these galls are relatively rare they are of minor importance.

Nematode galls in wheat often are mistaken for smut balls, cockle seeds, vetch seeds, bin-burnt kernels, ergot sclerotia, or other material commonly found in threshed grain. Galls are readily distinguished from smut balls by the fact that the latter are easily crushed between the fingers into a mass of black powder, whereas the galls are hard and firm. Cockle seeds should be recognized at once by their characteristic spiny seed coat and vetch seeds by their smoothness, rotundity, and uniform color. The ergot sclerotia formed in wheat heads by *Claviceps purpurea* (Fr.) Tul. can be identified by their color, shape, and cross section. Some of the differences between galls and other material commonly found in wheat are shown in Plate 5.

#### THE CAUSAL ORGANISM

The nematode disease often is called the eelworm disease of wheat on account of the eellike appearance and movements of the causal organism, *Tylenchus tritici*, as seen under the microscope. The mature gall contains a white fibrous mass of these microscopic worms. Their life history, according to Byars (6), is quite simple. Escaping into the soil from the disintegrating galls after the latter have been sown with the wheat, the nematodes reach the wheat seedlings, climb up the stem presumably, get in between the leaf sheaths, and finally reach the apical growing point of the culm, with which they are elevated when the culm elongates. Here they remain until the wheat head begins to develop, when in some manner they enter the floral organs which are stimulated to the formation of galls instead of some of the normal flower parts, such as the ovary and stamens. It is uncertain whether they enter the tissues by penetration or by inclusion. Within the newly formed galls they develop rapidly into mature males and females. Copulation and egg laying follow, and by the time the galls are mature they are filled with masses of newly hatched, partly dried larvae. Galls opened just before maturity are found to contain thousands of eggs from which these larvae develop. At a somewhat earlier stage are seen only the adult males and females.

The eggs are elongate, cylindrical bodies with the length about twice the diameter and granular in appearance. They vary somewhat in size, averaging about 40 by 85  $\mu$ . They have a single nucleus and a smooth tough chitinlike coat. The larvae emerge from the eggs a few days after these have been deposited, being quite fully developed while still in the uterus of the female. The freshly hatched larvae are about 500  $\mu$  in length, threadlike in appearance, and unable to withstand unfavorable conditions.

This so-called first stage is of short duration, and after one or more molts the larvae reach the second stage of their development. They remain in this stage until after they have escaped from the gall, have entered the tissues of another host plant, and have caused the formation of other galls. They vary in length from about 700 to 900  $\mu$  and in width from 15 to 20  $\mu$ , or about one-fortieth of their length. Their structure is very simple, consisting of a tube within a tube, the outer one being the body covering and the inner one the digestive tract. The anterior end is provided with a hollow buccal spear with which the organism probably pierces plant tissues and extracts nourishment after it has escaped from the gall and entered another plant. There is no sexual differentiation in this stage, but the beginning of the reproductive system appears as a half-moon-shaped, light area located midway between the ends of the intestine.

Investigators generally agree that the larvae take practically no nourishment until after they have invaded the tissues of the host plant. This belief seems to be borne out by the fact that as the free-living stage is prolonged the larvae become more transparent. The translucent granular matter in the intestine which is regarded as reserve food gradually disappears. Yet it seems reasonable to suppose that in order to produce such pronounced deformities in the plant before heading has begun the organism must use its buccal spear and esophageal bulb to puncture the cells and extract plant juices while still in the free-living stage.

After entering the host tissues the larvae rapidly develop into mature male and female adults. These are large enough to be clearly visible to the naked eye. The females average about 4 mm. and the males about 2.5 mm. in length. Both are proportionately wider than the larvae. The reproductive system in each is fully developed and occupies most of the body space, especially in the female. Egg laying continues for some weeks, each female being capable of depositing as many as 2,000 or more eggs during that period.<sup>5</sup>

The number of mature nematodes, as well as the relative number of males and females, varies widely in different galls. Green galls from spelt, wheat, and emmer were opened and the mature nematodes carefully counted. The results are shown in Table XII.

<sup>5</sup> For a technical description of eggs, larvae, and adults, see Department Bulletin 842 (6).

TABLE XII.—Number of adult nematodes found in immature galls from spelt, wheat, and emmer grown at the Arlington Experiment Farm in 1919

Source of galls.	Number of galls.	Highest number of—		Lowest number of—		Average number of—	
		Males.	Females.	Males.	Females.	Males.	Females.
Spelt.....	36	6	8	0	1	3	4.4
Wheat.....	40	18	14	2	3	7	8.0
Do.....	20	42	37	1	3	17	16.0
Emmer.....	94	40	45	2	3	20	22.0

These figures are larger than those of Marcinowski (17), who found that none of the nine galls examined contained more than 16 adults. Byars (6) states that a gall contains an average of six or seven females. According to the figures given in Table XII his estimates also are rather low.

#### SPREAD OF THE DISEASE

##### IN GRAIN, STRAW, AND MANURE

The most common and only important method of spreading this disease is by means of galls in the seed wheat from infested areas. It is likely to be spread from farm to farm by means of threshing machines. This was illustrated in Jackson County, Ga., where the disease occurred for many years only on those farms in a certain "threshing ring."

As many galls are blown into the straw at threshing time, this also may serve to distribute the disease, provided the straw is not allowed to decay in the barnyard or in the manure pit.

At Madison, Wis. (1921-22), the writer thoroughly mixed a quantity of barnyard manure with a generous sprinkling of whole galls. An equal quantity of manure was mixed with a like quantity of ground galls. This manure was stored in a manure pit for six weeks and then applied to two separate plats of land which later were sown to wheat. No infection of the wheat followed. Two adjacent plats of soil received an application of manure which was mixed with galls, both whole and ground, immediately before being spread on the land. Wheat sown on these plats showed abundant evidence of nematode injury in the fall and although badly winterkilled produced a number of infected heads the following summer. These results with others are shown later in Table XVII.

##### IN THE SOIL

The organisms may be spread in the soil to a limited extent by surface water, by infested soil on farm implements, or by the feet of farm animals going from infested to uninfested fields. In such instances the larvae may be within the galls or in a free-living condition in the soil. As they can endure long periods of desiccation they even may be blown about by the wind. However, dissemination by these means is so relatively unimportant that it is quite safe to grow wheat on clean soil adjacent to very badly infested soil. The writer has grown wheat repeatedly on plats a few feet away from badly infested soil, and where no heavy washing occurred was unable to find any infected heads in the plats on clean soil.

The free larvae are incapable of traveling very far by their own movements. Haberlandt (11) claims to have secured infection in wheat plants growing 20 cm. from the point at which the galls were placed in the ground. Byars (6) states that he "found abundant infection in wheat growing 30 cm. away from unopened galls." Marcinowski (17) secured no infection in plants growing beyond 5 cm. from the buried galls. Appl (1) states that the larvae can not reach plants 10 cm. from the galls.

The results bearing on this question, obtained by the writer during two seasons, were varied. Wheat was sown at Arlington Experiment Farm in the fall of 1919 in circular plats at different distances from the central area in which galls were placed 5 cm. below the surface of a sandy clay loam. A metal cylinder surrounded each plat to prevent washing. Wire cages were used to exclude birds and rodents, and every precaution was taken to prevent accidental spreading of the organisms. Infection was not secured in any plants growing beyond 10 cm. (4 inches) from the infested area.

TABLE XIII.—Effect of placing galls at different distances from the seed on the degree of infection in the heads of *Prelude* and *Marquis* wheats; Madison, Wis., 1922

Distance galls were placed from seed.	Heads of Prelude Wheat.					Heads of Marquis Wheat.				
	Exam-ined.	Infected.		Healthy	Shat-tered. <sup>a</sup>	Exam-ined.	Infected.		Healthy	Shat-tered. <sup>a</sup>
		Total.	Per cent.				Total.	Per cent.		
<i>Inches.</i>										
1.....	74	60	81	5	9	218	65	30	133	20
2.....	100	72	72	20	8	210	46	22	140	14
3.....	130	80	62	38	12	150	26	17	116	8
4.....	140	56	40	0	84	90	12	13	77	1
6.....	170	63	37	85	22	180	16	9	160	4
9.....	150	50	33	90	10	107	7	7	100	0
12.....	100	30	30	65	5	145	3	2	142	0

<sup>a</sup> Heads wholly or partly threshed by birds or by handling so that conclusive data could not be obtained.

This experiment was later repeated at Madison, Wis., spring wheat being used in this case in a black garden soil. Frames 4 feet square were sunk into the ground several inches and allowed to project above the surface about 2 inches. Within each of these frames and 4 inches from it was another frame sunk  $\frac{1}{2}$  inch into the ground and projecting  $\frac{1}{2}$  inch above the surface. On April 15, 1922, the area between these two frames was heavily inoculated with galls, whole galls in half of the space and coarsely ground galls in the other half. On April 21 *Prelude* wheat, a very susceptible spring variety, and *Marquis*, a more resistant variety, were sown in the center of the different frames at distances of 1, 2, 3, 4, 6, 9, and 12 inches from the infested outer area. A few kernels were sown in the inoculated soil to test the virulence of the inoculum. On May 15, about three weeks after sowing, abundant symptoms were observed in the *Prelude* plants 1 and 2 inches from the inoculated area, while all the plants growing in it were badly distorted. A few days later several plants 3 inches from the inoculum showed decided symptoms and upon examination were found to contain swarms of larvae. On June 5, or 45 days after sowing, 12 badly infested *Prelude* plants were found, 4 each at 6, 9, and 12 inches, respectively, from the inoculated area. When ripe, the plants in each frame were harvested and each



head threshed by hand. The results shown in Table XIII are conclusive evidence of the fact that the larvae can bring about infection when galls are placed in the soil as far as 12 inches (30 cm.) from the seed.

The larvae seem able to travel vertically as far as 30 cm. Marcinowski (17) buried galls at depths of 3, 6, 10, 15, and 30 cm., respectively. At the first four depths she secured head infection, the greatest being at 3 cm. At 30 cm. no galls were formed although a few larvae reached the plants.

The writer's results show greater movement of larvae than this, as he found that galls buried 30 cm. deep produced some head infection. Galls were buried at depths of 2, 4, 6, 9, 12, and 15 inches, respectively, in bottomless wooden frames sunk into the ground. In another series, boxes with closed bottoms were used to eliminate a possible complication due to water rising in the soil. Winter wheat was sown in these frames in October and in the following June the heads were collected and threshed by hand. The results are shown in Table XIV (A) and (B).

TABLE XIV.—Relation between the depth at which galls were placed in the soil and the resulting infection of wheat heads, Arlington Experiment Farm, 1919-20

A. IN BOTTOMLESS FRAMES

Depth (inches).	Number of heads.			
	Examined.	Healthy.	Infected.	
			Number.	Per cent.
2.....	120	70	50	<sup>a</sup> 42
4.....	90	60	30	33
6.....	40	30	10	25
9.....	66	53	13	20
12.....	60	54	6	10
15.....	56	56	0	0

B. IN FRAMES WITH CLOSED BOTTOMS

4.....	46	31	15	33
6.....	28	22	6	21
8.....	33	28	5	15
10.....	88	77	11	13

C. IN METAL CYLINDERS IN FIELD

2.....	12	6	6	50
4.....	15	9	6	40
6.....	22	14	8	36
8.....	28	19	9	32
10.....	25	20	5	20
12.....	30	27	3	10

<sup>a</sup> Heads somewhat shattered by birds.

Similar results were obtained in the field. Galls were buried at different depths in circular plats, and wire cages were used to keep out birds and rodents. Wheat was sown in the fall and when the heads were harvested the following June the results given in Table XIV (C) were obtained.

At Madison, Wis., in 1922, this experiment was repeated, but spring wheat was used. The galls, some of which were ground to insure early liberation of the larvae, were buried at the following respective distances

below the seed: 15, 12, 10, 8, 6, 4 and 2 inches, respectively. Three controls were sown. In one, galls were placed with the seed at the time of sowing; in another they were strewn on the surface of the ground immediately after sowing; and in a third no inoculum was used. With the exception of the three controls, the galls were placed in the soil on April 15, and the wheat was sown a week later. Three weeks after sowing, the seedlings in the two inoculated controls showed symptoms of nematode injury. Five weeks after the time of sowing, some infested plants were found in the frame in which galls had been placed two inches below the seed. No vegetative symptoms were observed in the plants in any of the other frames although galls were found in a number of the heads at harvest time, as shown in Table XV.

TABLE XV.—Percentage of infected heads of *Prelude* wheat resulting from placing galls in the soil at various depths in relation to the seed, Madison, Wis., 1922

Location of galls in relation to seed.	Heads of wheat.				
	Exam- ined.	Infected.		Healthy.	Shat- tered.
		Total.	Per cent.		
Control without galls .....	90	0	0	80	10
Galls on surface .....	80	49	61	26	5
Galls with seed <sup>a</sup> .....					
Galls 2 inches below seed .....	54	17	32	37	0
Galls 4 inches below seed .....	44	26	59	14	4
Galls 6 inches below seed .....	35	7	20	23	5
Galls 8 inches below seed .....	30	5	17	21	4
Galls 10 inches below seed .....	75	41	55	28	6
Galls 12 inches below seed .....	69	15	22	36	18
Galls 15 inches below seed .....	70	0	0	50	20

<sup>a</sup> Plants injured so badly in the seedling stage that no heads were produced.

Invasion by the nematodes probably occurred at so late a stage that head infection took place very shortly after the larvae reached the plants. It is evident from the high percentages of infected heads in the frames in which the galls had been buried 10 and 12 inches below the seed that, although some of the larvae are killed at the greater depths, many are able to travel upward through a foot of compact soil to reach the host plants. This fact proves the futility of deep plowing of the stubble as a control measure.

#### TRANSMISSION THROUGH BIRDS AND ANIMALS

To what extent the larvae of *Tylenchus tritici* survive passage through the digestive tract of birds and farm animals has long been a debated question. Marcinowski (17) fed galls to sparrows, goldfinches, pigeons, chickens, mice, gophers, and marmots. She collected and examined the excreta and found some live larvae in all except that from the chickens, gophers, and marmots. She succeeded in infecting wheat plants by placing the droppings of the goldfinches with the seeds sown. She concluded, however, that inasmuch as the birds took the galls very unwillingly and only when no other food was available, birds are a minor factor in the spread of the nematodes.

In 1921, E. R. Kalmbach, of the Bureau of Biological Survey, United States Department of Agriculture, working in cooperation with the writer, fed galls containing adults' eggs, and larvae of *Tylenchus tritici*

to two lots of sparrows. At various intervals of time after feeding, the feces of the first lot were examined and some of the birds in the other lot were killed and the contents of the digestive tract examined. Very rarely were any live larvae found in the feces or near the end of the alimentary tract and then only in very small numbers. It was concluded from this that birds are relatively unimportant in spreading the nematode disease.

In the nematode-infested districts it is a common practice to feed the gall-infested wheat screenings to farm animals. An experiment was conducted in cooperation with the Wisconsin Agricultural Experiment Station, at Madison, Wis., in 1921-22 to determine whether the organisms survive passage through the digestive tract of farm animals and remain capable of attacking wheat grown on land to which the manure is later applied. Two horses, two cows, eight hogs, four sheep, and twelve chickens were used in the experiment. In each case the different kinds of animals were divided into two groups. The first group received about a pint of whole nematode galls mixed with its daily feed. The second group received an equal quantity of ground galls. Small quantities of the manure from each of the first groups of animals were examined for the presence of galls but only in the excreta of the horse, cow, and hog were any found (Table XVI). The recovered galls were examined and nearly two-thirds of them were found empty while the rest contained only dead larvae, most of which were in the first stages of decomposition. This seemed to indicate that the nematodes failed to survive passage through the alimentary tract of these animals.

TABLE XVI.—Number and condition of galls and larvae voided by farm animals fed whole galls, Madison, Wis., 1922

Animal.	Number of galls.			Condition of larvae.
	Recover- ed.	Full.	Empty.	
Horse.....	70	30	40	Dead.
Cow.....	16	70	6	Dead.
Hog.....	36	3	33	Dead.
Sheep.....	0			
Chickens.....	0			
Total.....	122	43	79	

The feeding was continued for a week. The manure from the horses, cows, and hogs was collected daily and stored in separate heaps. The chicken manure was collected from the dropping board at the end of the week and divided into two lots: One lot was stored in a box for six weeks and the other lot was immediately applied to the land. The sheep manure was of little value in this experiment as nematode-infested feed became scattered about the sheep stall by the animals while feeding. The manure from the different animals in each lot was spread upon small plats and spaded into the soil. Turkey wheat was then sown on these plats on September 7.

Four control plats also were sown. On the first, uninfested manure was applied and uninfested seed was sown. On the second, uninfested manure was applied and infested seed sown. On the third plat barnyard manure artificially infested with galls was applied and uninfested seed was sown.

The fourth plat was like the third except that the galls were ground. During the fall the plants on all the plats were examined periodically for symptoms of the nematode disease. The plats which had received an application of artificially infested manure and also those in which infested seed had been sown produced a stand of wheat which was badly diseased. The plats upon which the sheep manure had been applied also produced numerous diseased plants due, as stated before, to the sheep scattering the infested feed about the stall. In no other plat were symptoms of the disease observed, and the examination of numerous seedlings from these plats under the microscope failed to reveal the presence of any larvae between the leaf sheaths of the plants.

During the winter, much of the wheat was winterkilled, especially in the three control plats which had shown a large amount of seedling injury in the fall. The wheat which survived the winter was harvested, hand-threshed, and the grain examined for galls. The results are given in Table XVII.

TABLE XVII.—Weight of grain and number of galls in hand-threshed grain from different plats of soil sown with Turkey wheat after receiving an application of manure from one of several kinds of farm animals fed whole or ground galls, or an application of manure to which had been added whole or ground galls and which was either immediately applied to the land or stored in a manure pit for six weeks, together with similar data from two control plats which had received an application of uninfested manure, one being sown with infested seed and the other with uninfested seed, Madison, Wis., season 1921-22.

Plat No.	Source and treatment of manure.	Kind of inoculum used.	Weight of grain.	Number of galls in grain.
			<i>Grams.</i>	
1	Barnyard.....	None.....	125	0
2	do.....	Galls in seed.....	25	47
3	do.....	Galls in manure.....	20	38
4	do.....	Ground galls in manure.....	15	30
5	Stored in pit six weeks.....	Galls in stored manure.....	200	0
6	do.....	Ground galls in stored manure.....	150	0
7	Horse.....	Whole galls in feed.....	300	0
8	do.....	Ground galls in feed.....	200	0
9	Cow.....	Whole galls in feed.....	700	0
10	do.....	Ground galls in feed.....	700	0
11	Hog.....	Whole galls in feed.....	1,400	0
12	do.....	Ground galls in feed.....	650	0
17	Cow.....	Whole galls in feed.....	650	a 88
18	do.....	Ground galls in feed.....	950	a 1
19	Sheep.....	Whole galls in feed.....	450	b 260
20	do.....	Ground galls in feed.....	200	b 120
21	Chicken, stored.....	do.....	550	a 12
22	Chicken, fresh.....	do.....	500	0
23	Chicken, stored.....	Whole galls in feed.....	600	0
24	Chicken, fresh.....	do.....	550	0

a Infection due to galls or larvae washing downhill from an adjacent plat intentionally inoculated at time of sowing.

b This infection was due to nematode-infested feed being scattered about the stalls by the sheep while feeding.

With the exception of a few cases of what were undoubtedly accidental infections (fig. 2), the plants from the plats which had not been purposely inoculated produced healthy heads. It may be safely concluded from these data that there is little or no danger of spreading the nematode disease by means of the manure from farm animals which have been fed infested grain. However, if gall-infested straw, used for bedding, is spread upon the land without first being stored in the barnyard or manure pit, it is quite probable that the land would become infested. In that case, however, corn or some other nonsusceptible crop could be grown with safety.

1 CLEAN SEED AND CLEAN MANURE		2 INFESTED SEED AND CLEAN MANURE	
3 GALLS IN FRESH MANURE		4 GROUND GALLS IN FRESH MANURE	
5 GALLS IN STORED MANURE SIX WEEKS		6 GROUND GALLS IN STORED MANURE	
7 MANURE FROM HORSE FED WHOLE GALLS		8 MANURE FROM HORSE FED GROUND GALLS	
9 " " COW " " "		10 " " COW " " "	
11 " " HOGS " " "		12 " " HOGS " " "	
13 UNINFESTED SEED		15 SEED WITH 15% GALLS BY VOLUME	
14 SEED WITH 5% GALLS BY VOLUME		16 " " 45% " " "	
17* MANURE FROM COW FED WHOLE GALLS	18* MANURE FROM COW FED GROUND GALLS	19 MANURE FROM SHEEP FED WHOLE GALLS	20 MANURE FROM SHEEP FED GROUND GALLS
21 MANURE FROM: CHICKENS FED GROUND GALLS (STORED)			
22 " " " " (FRESH)			
23 " " WHOLE " (STORED)			
24 " " " " (FRESH)			

\* SLIGHT INFECTION DUE TO WASHING FROM PLAT 14

FIG. 2.—Diagram showing the arrangement of the field plats in which were conducted the experiments, referred to in Tables III and XVII, concerning the transmission of nematodes through animals, their longevity in stored manure and the effect of sowing different amounts of galls with the seed at Madison, Wis., September, 1921, the slope of land being in the direction of the numerical progression in diagram; that is, plats 1 and 2 were the highest, and plat 24 the lowest.

## OVERWINTERING OF THE PARASITE AND THE TIME AND METHOD OF INFECTION

A considerable difference of opinion exists among investigators as to the time of year when the larvae leave the galls and invade the host plants. Roffredi (21) and Henslow (12), two of the earlier workers, believed that this takes place in the spring only, and that the larvae spend the winter within the galls in the soil. Marcinowski (17) likewise maintains, from the results of rather extensive experiments, that most of the larvae remain in the galls until spring. Some of the nematodes, she admits, may escape from the galls and invade the plants in the fall, but most of these return to the soil at the approach of cold weather. However, it appears that she based her conclusions upon the examination of relatively few plants and galls.

The writer agrees with Davaine (9) that under favorable conditions of moisture and temperature most of the larvae leave the galls in the fall within a few weeks after sowing and spend the winter between the leaf sheaths of the host plants. These conclusions are based upon the examination of large numbers of diseased seedlings throughout the fall and winter. These seedlings were obtained from plats in which gall-infested seed had been sown in the fall. Most of the 30 varieties of wheat sown showed abundant symptoms of invasion by the nematodes shortly after sowing, so that, in the opinion of the writer, there can remain no doubt of the fact that a general invasion of the plants by the nematodes occurs in the fall.

Some of the galls, however, retain their larval content all winter, as was shown by the examination of a number of them which had been buried in the soil in October and were dug up in the following spring. The numbers of full and empty galls and the length of time they were in the ground are shown in Table XVIII.

TABLE XVIII.—*Number of full and empty galls found in the ground in spring, in soil inoculated the previous fall, Arlington Experiment Farm, 1920*

Date.		Days in soil.	Number of galls.			
Sown.	Examined.		Examined.	Full.	Partly empty.	Empty.
Oct. 28, 1919.....	Mar. 17, 1920	140	22	17	3	2
Do.....	.....do.....	140	14	11	2	1
Do.....	.....do.....	140	16	12	2	2
Oct. 20, 1919.....	Mar. 25, 1920	156	37	15	4	18
Do.....	Apr. 9, 1920	171	39	5	7	27
Sept. 26, 1919.....	June 2, 1920	268	16	0	0	16
Total.....			144	60	18	66

That more empty galls were not found in the spring was due, undoubtedly, to their having disintegrated to such an extent that they were no longer distinguishable. The writer repeatedly has found wheat seedlings containing immense numbers of larvae from 10 to 15 days after gall-infested seed had been sown. From these facts we may conclude that the length of time it takes for the galls to disintegrate sufficiently to liberate the larvae varies considerably, owing probably to several factors, among which are temperature, moisture, and the thickness of the gall walls.

Although a relatively high temperature seems to be conducive to the opening of the galls, it is not favorable for infection. High temperature causes such rapid growth of the plants that before the larvae can effect a general invasion the most susceptible period of the plant has passed. Wheat plants grown in a warm greenhouse from infested seed show little infection, while in the cooler soil of the field, a general invasion of the plants usually occurs. Experiments in soil-temperature tanks indicate that infection occurs more readily at 12° and 16° C. than at 20°, 24°, and 28°. Prelude wheat was sown in the field at Madison, Wis., in July, 1921, and the soil was heavily inoculated with galls. The mean soil temperature for the four weeks after sowing was 80° F. (26.6° C.). The plants grew to maturity and produced healthy heads. However, when sown in cold soil in early spring and inoculated with galls, Prelude wheat produced practically no uninfected heads. This affords additional evidence that temperature is an important factor in infection.

The nematode larvae seem unable to invade the plant before the coleoptile has begun to unclasp or loosen about the young stem. Hundreds of wheat seedlings from heavily infested soil were examined and in no case were larvae ever found while the coleoptile was still firm about the base of the plant. But plants with this primary sheath slightly loosened, frequently contained larvae in abundance. Often they were found just within the loosening coleoptile and occasionally at the edge as though they were in the act of entering. At a slightly later stage, they were invariably found between the leaf sheaths near the growing point.

How long after the loosening of the coleoptile it remains possible for nematode larvae to invade the plant and bring about infection remains to be determined. Sommerville (23) sowed wheat in pots November 8 and kept them in a "cold greenhouse" (he does not state at what temperature) until December 16 when he placed them in the open. On April 4 he added 20 "cockles" (galls) to each pot. The wheat was harvested August 5 and "where the addition of 'cockles' was delayed for five months after planting, infection was practically as complete as when 'cockles' were sown with the seed." Marcinowski (17) claims to have secured infection by spraying a water suspension of larvae on plants of considerable size.

The writer placed a suspension of active larvae at the base of plants 6 to 8 inches tall, 16 days after sowing in a warm greenhouse, and 9 days later pronounced symptoms were observed. Likewise unopened galls placed near the plants 12 days after sowing produced characteristic symptoms of nematode injury 30 days later. From these and other observations, it may be tentatively assumed that the period during which the nematodes can invade the plant and bring about infection extends from the loosening of the coleoptile to the elongation of the culm, that is, as long as the terminal growing bud or embryonic spike is located near the base of the plant. Larval suspensions injected into the flowers at various stages of growth from the time the plants were still in the boot till they were in full flower produced no infection.

#### LONGEVITY AND VITALITY OF THE ORGANISM

##### LONGEVITY

Within the protective galls, the larvae may retain their vitality for many years. According to Néedham (18), Baher in 1771 succeeded in reviving larvae from galls that had been in the laboratory 27 years.

However, the movement Baher observed may have been the mechanical straightening of the dead larvae due to their absorption of water. Byars<sup>6</sup> proved that larvae may live within the galls for at least eight years. He examined the contents of galls from various countries and secured the data shown in Table XIX.

TABLE XIX.—*Reactivation of larvae from galls received from various countries*

Source.	Year received.	Date examined.	Age.	Percentage alive.
			Years.	
India.....	1914	Apr. 11, 1918	4	50-85
Russia.....	1912	.....do.....	6	50-75
China.....	1911	.....do.....	7	1-2
Turkestan.....	1910	.....do.....	8	10-15
China.....	1910	Mar. 26, 1918	8	0

These figures indicate that the larvae in the galls gradually die in the course of a few years. This grain, however, at some time or other may have been exposed to unfavorable conditions, such as high temperatures, and this would account for the small percentage of larvae found alive in the galls after eight years. Most investigators believe that they remain alive for a longer period.

Outside of the protective galls the larvae may exist either in a dried dormant condition much as when within the galls, or in an active, free-living condition in moist soil or between the leaf sheaths of plants. In the former state they remain capable of resuscitation after a considerable period, if kept dry and at a low or moderate temperature. The writer has revived larvae from galls that had been ground four years previously and kept in a glass jar in the laboratory.

#### DESICCATION

The larvae may be killed by such extreme desiccation as obtains in an ordinary desiccator containing calcium chlorid and sulphuric acid. This is especially true of those larvae which have been in a free-living state for some time. The writer placed larvae from galls and from wheat and rye seedlings in two series of watch glasses. These were placed in a desiccator and at regular intervals water was added to one of the watch glasses from each series in an attempt to resuscitate the larvae. Those taken from between the leaf sheaths of seedlings showed no indication of reviving in water after having been in the desiccator for two weeks. Those taken directly from the galls, however, were revived in water after more than two months of desiccation.

In an active, free-living condition the larvae can retain life for a considerable period. In sterile, distilled water they remained active for nearly five months. In infested soil and in an ectoparasitic state in plants larvae may live outside the galls for at least nine months. For instance, when wheat is harvested in June many galls fall to the ground. The larvae undoubtedly escape from these within a month. If clean seed is sown on this infested soil in October plants will be found with numerous larvae between the leaf sheaths in the following April or May.

<sup>6</sup> Unpublished data.



The larvae are quite transparent by this time, having used up much of the reserve food material in their bodies, but they are still alive and active after having been in a free-living state for 9 or 10 months. It has been found, however, that they do not survive in the soil long enough to infect a subsequent wheat crop after a nonsusceptible crop has occupied the land for a year. Neither will land infested at harvest time produce pronounced infection in spring wheat sown the following April.

#### LOW TEMPERATURE

Investigators differ as to the minimum temperature that can be endured by the larvae. Marcinowski (17) states that larvae between the leaf sheaths of plants are killed at a temperature of  $-14^{\circ}$  C. Davaine (9) claims that larvae can endure a temperature of  $-20^{\circ}$ , and Pennetier (20) also states that he exposed larvae to a temperature of  $-20^{\circ}$  for five hours or more without killing them. Neither states whether the larvae were within the galls or in a free-living condition.

The writer exposed soaked galls to a temperature of  $-40^{\circ}$  C. for five minutes. The galls were then opened in water and the larvae were observed to move actively within a few hours. A section of a ryeseedling stem containing larvae was similarly exposed to  $-40^{\circ}$  for five minutes and then placed in tap water. The larvae remained straight and at no time during the 40 hours following did they show any indication of life, while larvae from another section of the same stem not exposed to the cold continued active movement. This seems to show that larvae taken directly from the galls can endure cold, as well as desiccation, to a greater degree than larvae that have been in a free-living state for some time. This undoubtedly explains the difference in the findings of Marcinowski (17) and those of Davaine (9) and Pennetier (20).

#### HIGH TEMPERATURE

Byars' (6) experiments on the effects of high temperature on the larvae both within and outside the galls showed that in previously soaked galls larvae are killed by immersion in water at 50, 52, 54, and  $56^{\circ}$  C. for 30, 20, 10, and 5 minutes, respectively. In galls not previously soaked in water a longer time is required. Free larvae placed in water at the above temperatures succumbed in half the time required to kill those inside the soaked galls. It happens that the same temperature fatal to nematodes in the galls is effective in controlling loose smut of wheat, so that if seed wheat in the infested region is treated for the latter disease the nematodes, if present, also are killed.

#### CHEMICALS

It has been found that any chemical able to penetrate the thick wall of the gall and kill the larvae inside will also kill the embryo of seed grain. Therefore, any attempt to control the nematode disease by treating the seed with chemical disinfectants is impracticable. Even when outside of the gall, the tough body wall of the nematodes makes them extremely resistant to the toxic action of chemicals.

Pennetier (20) states that the free larvae can endure 0.5 per cent sulphuric acid for two hours, 25 per cent alcohol for six hours, and glycerin for one month. Byars (6) found that nearly five hours in for-

maldehyde of 1:240 strength, or four and a half hours in mercuric chlorid of 1:1000 strength, were required to kill the free larvae. They endured immersion in 0.5 per cent sulphuric acid for three hours, and 5 per cent solution of copper sulphate for six and one-half hours. Within the protective galls, of course, the larvae can endure a much longer period in any of the above chemicals.

Sommerville (23) tried various strengths of copper sulphate, sulphuric acid, and commercial formalin, but found that whatever affected the nematodes also reduced the germinating power of the seed. He concludes that seed treatment is impracticable.

### CONTROL MEASURES

Inasmuch as seed treatment is inadvisable, and completely resistant varieties unknown, the control measures are limited mainly to sanitation and rotation, in short, clean seed sown in uninfested soil.

### CLEAN SEED

The best way to insure having suitable clean seed is to obtain a supply that has been grown on uninfested land, preferably in the same or a near-by locality where the disease does not exist. If this is impossible, the gall-infested seed on hand may be cleaned by the salt-brine method devised by Johnson and Vaughan (14) for removing ergot from rye. This consists of pouring the infested grain into a 20 per cent solution of common salt (sodium chlorid), stirring vigorously in the meantime, and then skimming off the galls, which float, due to their lower specific gravity, while the sound wheat sinks. The grain should then be rinsed in fresh water and spread out to dry. It is best to sow it at once. If, in addition to this treatment, the grain be soaked in water at 54° C. for 15 minutes after a preliminary soaking in cold water for several hours, any galls that may not have been removed will be rendered harmless, and, according to Humphrey and Potter (13), loose smut also will be prevented. This latter process, however, is one which demands great care, as there is danger of injuring the vitality of the seed by excessive temperature or too long an exposure.

### CLEAN SOIL

In order to free soil from the wheat-nematode infestation it is necessary to keep susceptible crops off the land for one year. This was demonstrated by rotation experiments conducted on infested soil at Morrisville and Woodstock, Va., as shown in Table XX.

TABLE XX.—Arrangement of rotation plats at Woodstock and Morrisville, Va., showing the crops grown on each plat in each season for four years, on land badly infested in 1917, and the condition of the wheat following the different rotations

Year.	Plat No. 1.	Plat No. 2.	Plat No. 3.
1917.....	Wheat (infected).....	Wheat (infected).....	Wheat (infected).
1918.....	Grass.....	do.....	Do.
1919.....	Corn.....	Grass.....	Do.
1920.....	Wheat (uninfected).....	Wheat (uninfected).....	Do.

Soil on which badly diseased wheat had been grown in 1917 was divided into three separate portions, each of which was given a different rotation. The first part was in grass in 1918, corn in 1919, and wheat in 1920. The second part was in wheat in 1918, grass in 1919, and wheat in 1920. The third part was used for wheat in all three years. In each case the wheat grown in 1920 on the rotated fields was entirely free from the disease, while that grown on the second plat in 1918 and on the third plat in all three years was badly infected, although clean seed had been used. Similar results have been obtained on small plats at Arlington Experiment Farm and on the Agricultural Experiment Station Farm at Madison, Wis.

Marcinowski (17) advises the "catch crop" method suggested by Kühn (16). This consists in growing a susceptible cereal on the infested soil, allowing the plants to become thoroughly infested with the nematodes and then destroying the plants. This plan is both ineffective and impractical, because all the nematodes do not enter the seedlings and the cost involved is prohibitive. Inasmuch as rotation and clean seed offer such a simple remedy it would be manifestly unwise and unprofitable to attempt any uncertain method.

#### RESISTANT VARIETIES

Among the many varieties of wheat tried in experiments covering three years, none showed sufficient resistance to nematode infection to be of any value in controlling the disease. Kanred showed marked resistance, but, nevertheless, enough galls were produced to perpetuate the disease. A wheat variety, to be effective in nematode disease control, would have to be practically immune.

#### SUMMARY

The nematode disease of wheat has been reported from all continents. In the United States it appears to be confined to Virginia, West Virginia, North Carolina, South Carolina, and Georgia.

Severe losses occur where the disease is prevalent, some fields being damaged as much as 50 per cent. Preliminary field data indicate that spring wheat is severely injured if infested seed is sown. Fortunately, the disease does not occur in any spring-wheat district in this country.

The disease attacks wheat, rye, emmer, and spelt with almost equal virulence. Oats and barley, while capable of being parasitized to some extent, are practically immune, as are also the various grasses.

In the seedling stage the disease causes wrinkling, twisting, or various other distortions of the leaves. The emerging leaf often is held by the older one, thus causing a "buckling" of the former while still in the inclosing sheath. Infected plants are usually shorter and thicker than normal ones. Badly infested seedlings often wilt and die.

In the mature heads the disease is characterized by the presence of hard, dark galls in place of normal kernels. These galls are somewhat thicker than wheat kernels and cause the glumes to stand out as in bunt-infected heads.

Several galls of varying size may be produced in one head. Galls in rye generally are smaller and lighter colored than those in wheat. They also are less conspicuous in the head and do not shatter out as easily.

The causal organism (*Tylenchus tritici*) is a minute nematode and may be found in great numbers in the larval state inside the mature galls. From 2,000 to 20,000 or more larvae are found in one gall. They are slender, threadlike, round worms nearly 1,000  $\mu$  in length and 25  $\mu$  in width. Escaping from the disintegrating gall into the soil, they reach the growing point of the host plant where they remain ectoparasitically until the flower parts begin to form, when they cause the formation of galls in place of kernels. Inside of these galls the larvae develop into male and female adults. Each female may produce several thousand eggs, which in a short time hatch into larvae. These larvae go into a dry, dormant state in the mature gall, and in this condition they can remain alive more than 10 years. They can endure extremely low temperatures and considerable desiccation, but immersion in water at 56° C. for 5 minutes kills them. They are resistant to chemicals, so that the latter are ineffective in seed treatment in concentrations that will not injure the seed. Although not so resistant to unfavorable conditions when outside of the protective galls, nevertheless in a dried dormant condition they are able to remain alive for several years. Larvae that have been in the free-living state for some time, in the soil or between the leaf sheaths of plants, succumb more readily to unfavorable conditions than those remaining in a dried dormant condition.

The disease is most commonly spread by means of infested seed and straw. It may be carried from one farm to another by the threshing machine. Screenings from infested wheat may also carry the disease to a new area. Other possible agencies of dissemination are farm implements, the feet of animals moving from infested to clean soil, barnyard manure from infested straw, and running surface water. Birds are of minor importance as agencies of distribution.

Through their own efforts, the larvae were unable to effect a general invasion of the wheat at a distance of more than 4 inches (10 cm.) laterally although a few reached plants 12 inches (30 cm.) distant. Vertically, they moved from 12 inches below the seed and produced infection.

The organisms invade the plant after the loosening of the coleoptile, and presumably invasion may continue until elongation of the culm occurs.

The organisms may overwinter within the protective galls or in a free-living state in the soil or between the leaf sheaths of the host plants.

The disease is easily controlled by the use of clean seed sown on clean soil. Susceptible cereals should be kept off infested fields for at least one year, as this will starve the nematodes in the soil.

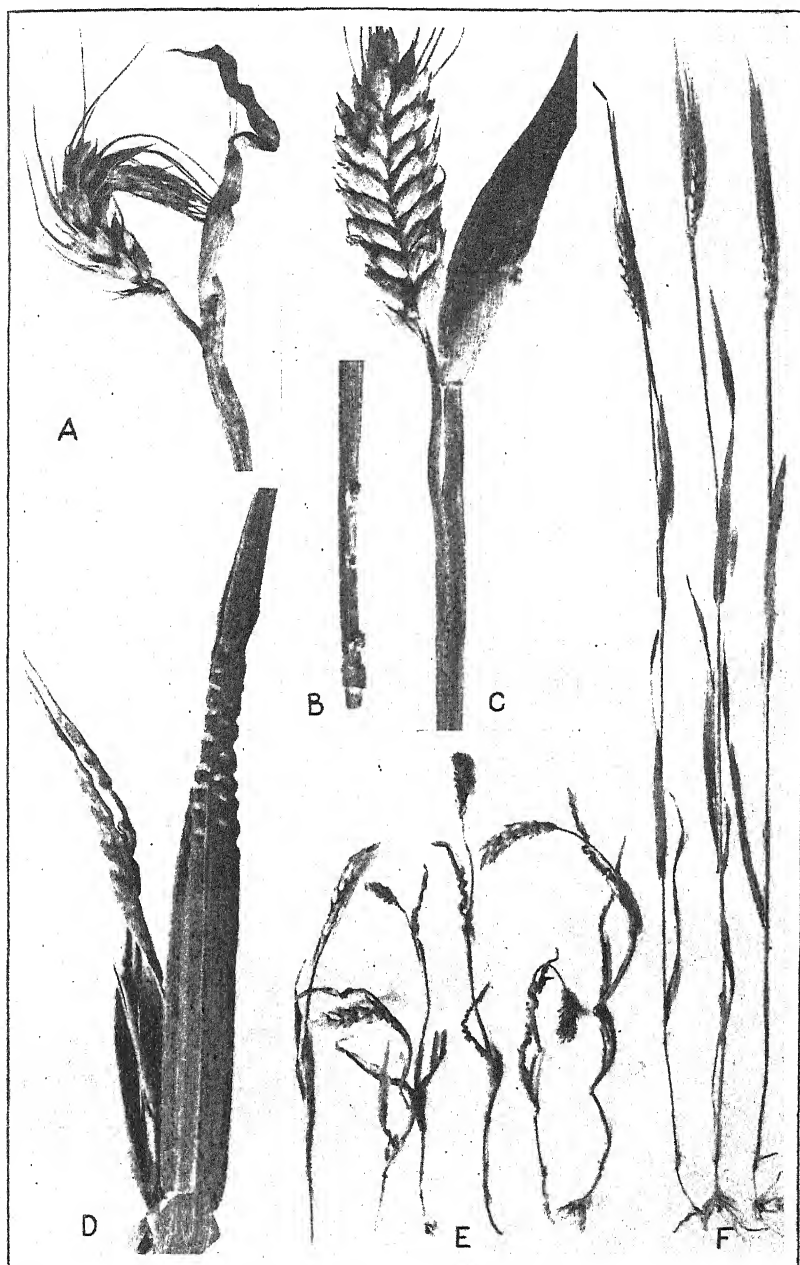
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PLATE I

- A.—Head of nematode-infected Nebraska Hybrid wheat. The badly distorted flag leaf is interfering with the natural emergence of the head from the boot.
- B.—Galls on the stem of Black Winter emmer.
- C.—Gall on the leaf of Black Winter emmer.
- D.—Leaf markings, an early symptom of the nematode disease. Some of these show slight discoloration, giving a mottled appearance to the leaf. The split leaf shows a more advanced stage of this symptom.
- E.—Prelude spring wheat affected by the nematode disease due to sowing gall-infested seed.
- F.—Healthy Prelude spring wheat grown from uninfested seed sown at the same time.



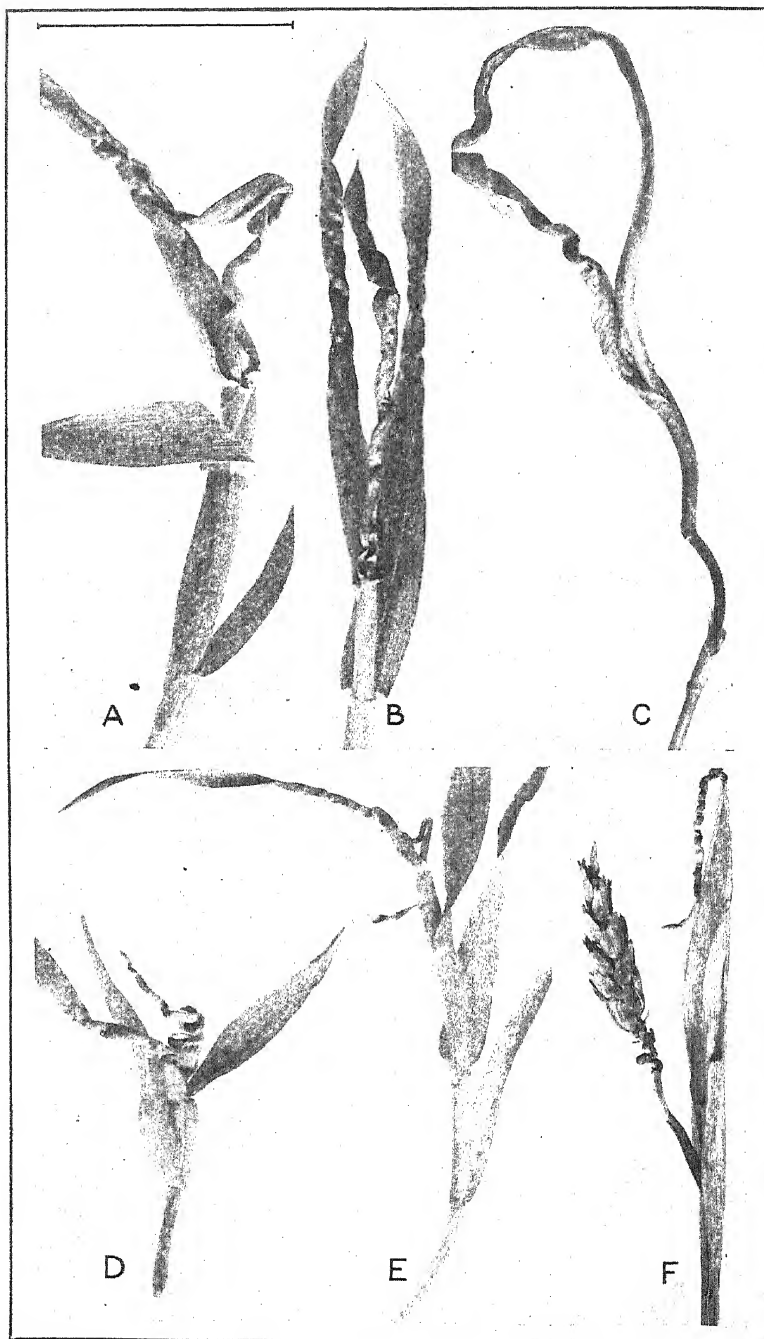




PLATE 2

- A.—Wrinkling and curling of the leaf edges toward the midrib in a nematode-infected wheat plant. This is a typical case of the emerging leaf being held by the curling of the older leaf enclosing it.
- B.—Twisting of the leaves of a wheat plant due to the nematode disease. The slight natural clockwise twist of the leaf is often severely aggravated in infected plants.
- C.—A pronounced case of bending of the culm of a nematode-infected wheat plant, due to successively emerging leaves being held by the older leaves as shown in A. The lower leaves have been removed in order to disclose the crooked stem.
- D and E.—Badly distorted seedlings of Red Rustproof oats found on dissection to be infested with vast numbers of nematodes about the growing points.
- F.—One of a large number of wheat plants found near Ocean View, Calif., in 1920, and showing symptoms similar to those caused by nematodes but which are due to climatic or other environmental conditions.

PLATE 3

- A.—Seedling of Hansee Hull-less barley infested with nematodes as shown later by dissection under the microscope. Note slight wrinkling of the leaf and bulging of leaf sheaths at base of plant. The symptoms are not so pronounced as those of infested wheat seedlings.
- B.—Heads of Hansee Hull-less barley infected with nematodes. These heads contained characteristic nematode galls and also a few kernels.
- C.—Head of Genesee Giant wheat uninfected.
- D.—Heads of Genesee Giant wheat infected with the nematode disease. Note the spreading glumes, the distorted awns, and the dark galls in place of kernels.
- E.—Head of Von Rümker rye uninfected.
- F.—Head of Von Rümker rye infected with the nematode disease. Note the greater prominence of galls near the base of the spike.

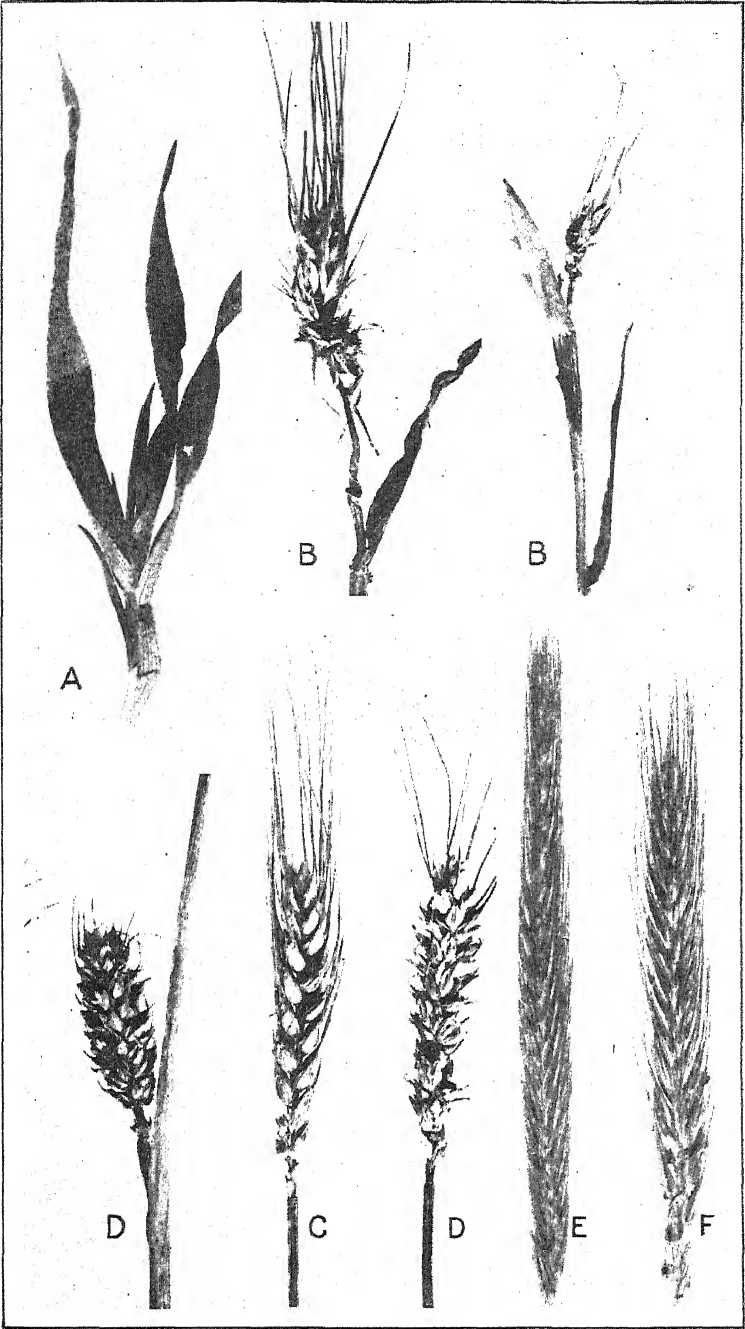




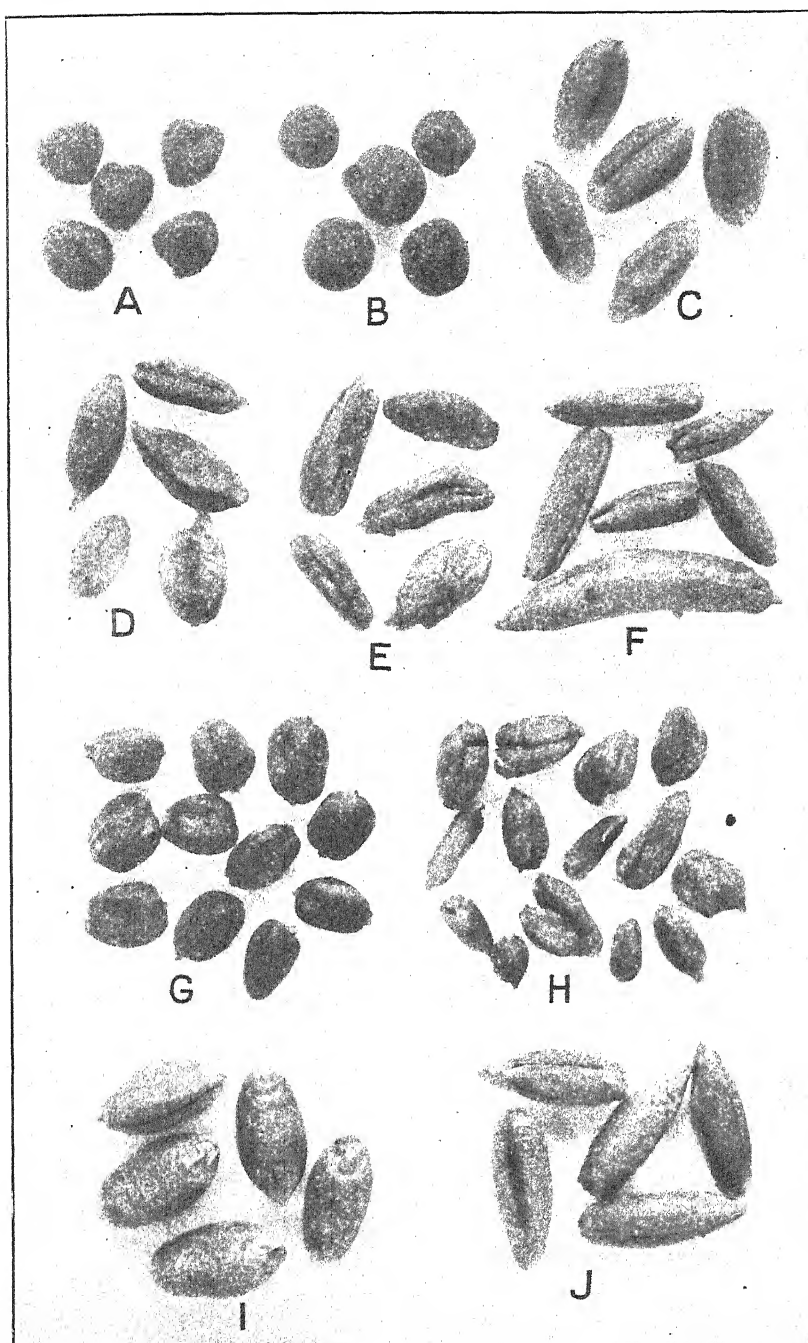
PLATE 4

- A.—Wheat infested with the nematode disease. When this wheat was sown, nematode galls were sown with the seed. Note the severe stunting of the plants and the wrinkling of the leaves.
- B.—Barley growing in a row adjacent to the badly diseased wheat and similarly inoculated but apparently not affected by the nematodes.

## PLATE 5

Comparison of nematode galls from wheat and rye with normal kernels of wheat and rye and with impurities commonly found in threshed grain. Note the comparative uniformity in the size and form of the galls from wheat and the variation in the size and form of the galls from rye.

- A.—Cockle seed.
- B.—Vetch seed.
- C.—Bin-burnt wheat kernels.
- D.—Bunt balls.
- E.—Ergot sclerotia from wheat.
- F.—Ergot sclerotia from rye.
- G.—Galls from wheat.
- H.—Galls from rye.
- I.—Wheat kernels.
- J.—Rye kernels.







## CORN ROOTROT STUDIES<sup>1</sup>

By THOMAS F. MANNS, *Plant Pathologist and Soil Bacteriologist*, and CLAUDE E. PHILLIPS, *Delaware Agricultural Experiment Station*<sup>2</sup>

During the past few years a great deal of study has been devoted to the corn rootrot disease. Most of the investigators agree more or less on the symptoms manifested by it, but there seem to be differences of opinion as to the relative importance of the organisms causing it. The report which follows presents the results of investigations made to determine the importance of the various causal organisms. Time limitations made it necessary to conduct all of the infection work under laboratory and greenhouse conditions.

### HISTORICAL REVIEW

A review of the literature covers investigations pertaining to corn rootrot in so far as the writers have been able to secure them. In this review special mention is made only of results which bear directly on the importance of the causal organisms.

Saccardo (11, p. 14)<sup>3</sup> reported *Oospora verticilloides* in *Zea mays* in 1886. It is now quite certain that this fungus is identical with Sheldon's (13) *Fusarium moniliforme*. Burrill and Barrett (2) in 1909, investigating the earrots of corn, reported that—

the active agents of destruction are several species of parasitic fungi, among which one does by far the most damage, probably 90 per cent of the whole amount. This is known botanically as *Diplodia zeae* (Schw.) Lev. \* \* \* At least three other species of fungi all belonging to the genus *Fusarium* attack, with somewhat similar results, the developing ears of corn.

Heald, Wilcox and Pool (7) in 1909 stated that—

*Diplodia zeae* produces in the ear a condition which may be called "dry rot," though probably the majority of corn growers refer to such ears as molded.

Stevens and Hall (15) in 1909 also reported that they found *Diplodia zeae* in North Carolina where the disease is known as "mold," "mildew," "rot," and "souring." Arzberger (1) in 1913, working upon cobrot of corn, stated that—

*Coniosporium* has an economic significance in that it destroys the cob tissue as a saprophyte; its effect on the kernels is rather limited when compared with the injury of *Diplodia*, *Fusarium* and other fungi.

Hoffer and Holbert (8) in 1918, found that—

the same organism which causes scab of wheat also causes rot of the stalks, ears and ear-shanks of corn plants.

Valleau (17) in 1920, reported that—

*Fusarium moniliforme* is an active parasite and is capable of causing root and stalk rots of corn under laboratory and field conditions. Inoculation experiments with

<sup>1</sup> Received for publication Nov. 21, 1923.

<sup>2</sup> The major part of this investigation was made by the junior author in preparation for a minor thesis in graduate work in plant pathology at the University of Delaware.

<sup>3</sup> Reference is made by number (italic) to "Literature cited," p. 964.

corn seedlings are of little value until a method can be devised of ridding the seed of contamination of organisms or until a source is discovered from which disease-free seed may be obtained. *Gibberella* sp. may or may not be present with root and stalk rots. *Fusarium moniliforme* appears to be the more active parasite when it and *Gibberella* are associated on rotting stalks of corn. Because of the high degree of infection with *F. moniliforme* over much of the corn belt, it will probably prove to be the most common cause of root and stalk rots of corn.

In 1921 he (18) reported that—

disease-free seed probably does not exist under average field conditions.

Manns and Adams (9) in June, 1921, in a popular bulletin, reported four parasitic fungi as very common in seed corn. These are *Cephalosporium sacchari*, *Gibberella saubinetii*, *Fusarium moniliforme* and *Diplodia zeae*. They stated that—

in this State (Delaware) corn is internally infected with four different parasitic fungi, to the extent of 39.54, 5.95, 19.92, and 5.69 per cent, respectively, for each of the diseases.

W. A. Taylor (16, p. 33-35), Chief of the Bureau of Plant Industry, United States Department of Agriculture, speaking in 1921 of the rots of corn, reported that—

the chief fungus parasites are *Gibberella saubinetii* (the wheat scab fungus), different species of *Fusarium*, especially *Fusarium moniliforme*, and *Diplodia zeae*.

Manns and Adams (10) in 1923 stated that—

a fungus unlike any previously reported in this country as far as we could determine was found very prevalent internal of seed corn. This fungus morphologically agrees with the description of *Cephalosporium sacchari* Butler, as reported by Butler and Khan (4) on sugar cane in India. Butler (3, p. 402-404) also found this fungus on sugar canes shipped from the United States to India. In view of these facts and because of the close relationship between the two hosts, it seems better to refer our fungus tentatively to this form than to create confusion by describing a new species. Further studies are in progress to determine the status of our fungus. The following four parasites were consistently found in our studies: *Cephalosporium sacchari* Butler; *Fusarium moniliforme* Sheldon; *Gibberella saubinetii* (Mont.) Sacc. and *Diplodia zeae* (Schw.) Lev. The parasitism of these fungi has been determined by inoculations. So far as we have been able to determine the fungus here referred to as *Cephalosporium sacchari* is reported for the first time as a parasite of corn. *Fusarium moniliforme* is considered identical with *Oospora verticilloides* described on corn in Italy by Saccardo.

Duddlestone and Hoffer (6) in 1921, reported that—

in a test of over 14,500 ears at Shelbyville, Ind., in 1920, 27 per cent showed serious infections of *Fusarium* sp. and *Diplodia*.

Sherbakoff (14) in his investigations found that in Tennessee, as in other States, the most common *Fusarium* of corn is *Fusarium moniliforme* Sheldon. Clayton (5) in 1922, reported that—

work done in Ohio during the winter of 1920 and 1921 showed that the fungus *Diplodia zeae* was very prevalent in seed corn.

Dr. J. F. Adams in an unpublished departmental report for 1921 (Delaware) on inoculation experiments on corn 4 feet high to the tasseling stage, with *Gibberella saubinetii*, *Diplodia zeae*, *Fusarium moniliforme* and the fungus tentatively referred to as *Cephalosporium sacchari*, notes the following:

The weakest lesions resulting from the field inoculations were found in the results of *C. sacchari* and *F. moniliforme* particularly on the younger corn. The most conspicuous lesions on the stalks were found with *G. saubinetii* and *D. zeae*. Internodal inoculations produced the most extensive lesions.

The literature as here reviewed shows that investigators have determined upon four different organisms as the principal ones associated

with corn rot diseases. These organisms are *Fusarium moniliforme* Sheldon; *Gibberella saubinetii* (Mont.) Sacc.; *Diplodia zeae* (Schw.) Lév., and *Cephalosporium sacchari* Butler. It is known that several of these organisms inhibit germination and appear to be active factors in the production of stalk and ear rots. Their importance as factors in causing rootrot, however, is still in dispute. It is even questioned whether soil reaction is not the real factor predisposing corn to the attacks of these so-called seedling and rootrot diseases.

#### EXPERIMENTAL METHODS

The value of an experiment such as the one herein reported depends to a great extent upon the care with which it is conducted and therefore the methods used will be described.

The nature of the infection carried in seed corn showing no external symptoms of disease was determined by germination. A representative sample was selected from each ear of corn, and the fungi carried internally were determined by the method described by Manns and Adams (10) as follows:

Fifteen or more kernels are disinfected in a test tube 150 by 20 mm. for one minute in a solution of 50 per cent alcohol containing 1 gm. of bichlorid of mercury in each liter. This solution is known as a 1 to 1,000 HgCl<sub>2</sub> in 50 per cent alcohol. Following this treatment the kernels are washed in the same tube with two successive washings with 20 cc. each of sterile water, and immediately 10 kernels are removed aseptically with sterile forceps and placed with the germ side down on 20 cc. of nutrient dextrose agar in a sterile culture dish. Further, 5 of the remaining kernels are each placed in a sterile culture dish, and with a sterile scalpel the point of the kernel, which is the portion that contains most of the internal infection, is cut off  $\frac{1}{8}$  to  $\frac{1}{4}$  inch from the end; then with a strong sterile forcep each point is placed in the mouth of a heavy-walled tube (it requires a strong tube and strong forceps, as crushing is not easy) 150 by 20 mm., containing 10 cc. of [liquefied] sterile nutrient dextrose agar medium at 43° C.; the point is thoroughly crushed and shaken down into the medium, then well mixed and poured into the sterile culture dish containing the remaining part of the kernel.

This method was used extensively by the senior author in his studies of flax diseases in 1904<sup>4</sup> and on wheat diseases in 1909 (12).

The following method of plating soil was used: One gm. of air-dry soil was placed in 100 cc. of sterile water. Ten cc. of this solution were by means of a sterile pipette placed in 90 cc. of water, making approximately a 1 to 1,000 solution. Ten cc. of the 1 to 1,000 solution were placed in another 90 cc. of sterile water, making approximately a 1 to 10,000 solution. One cc. of each solution was put in a test tube containing 12 cc. of weak nutrient glucose agar, mixed thoroughly, and poured into a sterile Petri dish. The plates were read 7 to 10 days later. To insure a fair degree of accuracy, six plates of both the 1 to 1,000 solution and the 1 to 10,000 solution were made and the average taken when read.

The method employed in growing the corn under sterile conditions has, so far as could be learned, never been previously reported. The jars used in this work were the ordinary one-quart Mason jars, although two-quart jars would have permitted better growth. Each jar was filled with a rich garden soil and sterilized from four to six hours at 7 to 10 pounds pressure in a steam autoclave. Plate cultures were run on several of the jars and they were found to be free of all organisms. The soil used was a rich compost such as was being used in the greenhouse by the horticultural department for potting. To get disease-free seed the surface-sterilized

<sup>4</sup> MANN, T. F. FUNGI OF FLAX SICK SOIL AND FLAX SEED. 1904. Unpublished manuscript (Master's thesis) filed in Department of Botany, College of Agriculture, Fargo, N. Dak.

corn was first germinated in Petri dishes (Pl. 1) on a weak nutrient glucose agar. Only seedlings found to be free from infection at the end of a five to six day period were used. It was discovered that disease-free seed was very difficult to secure, but in these investigations some corn seed samples were found which after surface treatment were practically 100 per cent disease free. The seedlings were transferred from the plates to the jars with sterile forceps. During this transfer some of the soil in the jars was exposed to the air in the laboratory, but all possible precautions were taken to prevent infection. The table on which the work was done, the hands, and all dishes and instruments used were swabbed with a 1 to 1,000 HgCl<sub>2</sub> in 50 per cent alcohol solution. At the time of planting, the soil in the jars was inoculated with the various organisms. This inoculation was obtained from pure cultures of these organisms grown on weak nutrient glucose agar. A sufficient quantity of the culture was added to the jars to insure a thorough inoculation. All experiments were run in duplicate. After the corn was planted the tops of the jars were covered with sterile cotton. It was necessary to exercise care when the plants were coming through to see that the cotton cover did not interfere with their growth. The jars were kept properly supplied with water; the optimum moisture condition was met by supplying 30 per cent moisture at the time of planting and keeping this replenished every other day.

#### STUDIES WITH ROOTROT SICK SOIL

Preliminary studies were made on two soils taken from adjacent fields on a near-by farm. Previous to the growing of the corn crop in 1921, both fields had been part of a peach orchard for 12 years. Field No. 1 produced much rootrot and fallen corn in 1921, while field No. 2 produced a good corn crop. It is not known by the writers what fertilizer or cultural treatments the fields had had during the 12-year period previous to the experiment, but it is not thought likely that a very high degree of fertility was maintained. Field No. 1 was the lower of the two fields, and was poorly drained. The soil was a fine sandy loam, light brown in color. Large areas of nut grass (probably *Cyperus rotundus*) were growing in this field. Field No. 2 was somewhat higher than No. 1 and was better drained. The soil was a fine sandy loam, very dark in color. These soils were plated and examined for pathogenic fungi; attention was given only to the four organisms, namely, *Fusarium moniliforme*, *Gibberella saubinetii*, *Diplodia zeae* and the so-called *Cephalosporium sacchari*. The soil from field No. 1 was found to be apparently free from the above organisms, while field No. 2 showed an average of 300 colonies of *F. moniliforme* per gram. The other organisms did not show in the cultures. Total nitrogen and total organic matter determinations were run on each of these soils. The results were as follows:

Nitrogen:	Per cent.
Field No. 1.....	0.040
Field No. 2.....	.046
Organic matter:	
Field No. 1.....	2.60
Field No. 2.....	2.96

The soil was brought into the laboratory from each of these two fields. The soil from field No. 1 (the nonresponsive sick field) was put into two 3-gallon pots labeled 1A and 1B. The soil from field No. 2 was put into similar pots and labeled 2A and 2B. Pot 1A received a treatment of 7½

gm. of calcium oxid in 15,000 gm. of soil; this is equivalent to 1,000 pounds of lime for a 2,000,000-pound acre (about a 6-inch acre); pot 1B received the equivalent of a treatment of 400 pounds of 2-8-6 fertilizer per acre, that is, 1 gm. sodium nitrate, 3 gm. 14 per cent acid phosphate and 1 gm. potassium chlorid in 15,000 gm. of soil; pot 2A received the equivalent of 500 pounds of lime per acre, that is, 3.75 gm. calcium oxid in 15,000 gm. of soil, and pot 2B received a treatment similar to pot 1B.

Corn which was apparently free from infection and which had been surface-sterilized according to the method previously given (p. 959), was planted, 15 kernels in each pot. The pots were planted on December 22, 1921; and on January 14, 1922, all plants except four in each pot were taken out. Several of the plants removed showed external evidences of infection and crushed cultures were made of the hypocotyl and lower part of the stem of each plant. No differences in the fungus floral content in any of the pots could be noticed. The plates showed large numbers of *Bacillus radicola*, several colonies of a *Fusarium* sp., a few colonies of *F. moniliforme* and one colony of *Cephalosporium sacchari*. On February 8, two plants were removed from each pot, examined and photographed. The examination showed that in pot 1A the hypocotyls were healthy. In pot 1B the hypocotyl of one plant was healthy and the other showed a pronounced lesion. In pot 2A the hypocotyl of one plant was dead and the other showed pronounced lesions. In pot 2B the hypocotyls were healthy. The work was discontinued here. The results obtained in the laboratory and greenhouse were exactly the reverse of the conditions found in the field, a fact which would seem to indicate that the "sick" condition of Field No. 1 was not due to the presence of pathogenic organisms in the soil. Just what the sick condition was caused by is still an open question. It might be the result of poor drainage or of some other purely physical condition.

#### INFECTION EXPERIMENTS WITH CORN ORGANISMS

The major part of these studies was concerned with trying to determine the effect of four different fungi upon corn, namely, *Fusarium moniliforme*, Sheldon; *Gibberella saubinetii* (Mont.) Sacc.; *Diplodia zeae* (Schw.) Lév.; and the fungus tentatively referred to by Manns and Adams (10) as *Cephalosporium sacchari* Butler. As has been previously stated, these are the fungi most commonly associated with corn ear, stalk, and root rots.

In order to reduce the probability of error the work was triplicated. The jars were handled according to the method previously described. The first series contained ten jars. Jars No. 1 and No. 2 were inoculated with *Diplodia zeae*; 3 and 4 with *Cephalosporium sacchari*; 5 and 6 with the organism tentatively referred to as *Cephalosporium sacchari* and with *Fusarium moniliforme*; 7 and 8 with all four of the fungi under consideration, and 9 and 10 were held as controls; that is, were not inoculated. These jars were planted on February 28 and were examined on March 17. The results on the latter date were as follows:

Jar No. 1.—Inoculated with *Diplodia zeae*. One plant dead and another almost dead. Some *Trichoderma koningii* found. Both plants contained large quantities of *Fusarium moniliforme* spores in the stems.

Jar No. 2.—Same as Jar 1 except that no *F. moniliforme* spores were found on the stems. Some, however, appeared on the roots.

Jar No. 3.—Inoculated with *Cephalosporium sacchari*. One plant dead and one equal to those in the control jars.

Jar No. 4.—One plant dead and one equal to the control plants. Both living plants looked fairly healthy. (One of these plants grew until about April 17 and the other grew practically to maturity and produced a small ear and a tassel about the last of May.)

Jar No. 5.—Inoculated with *Cephalosporium sacchari* and *Fusarium moniliforme*. Both plants dead. A considerable quantity of *Trichoderma koningii* was found on top and around the sides of the jar. The stems of the plants when examined showed *F. moniliforme*; there was fairly good root growth.

Jar No. 6.—Both plants were dead. The stem when examined showed *Fusarium moniliforme*. *F. moniliforme* was also found fruiting around the edge of the jar. There was good root growth.

Jar No. 7.—Inoculated with *Gibberella saubinetii*, *Diplodia zeae*, *Fusarium moniliforme*, and *Cephalosporium sacchari*. Both plants were dead.

Jar No. 8.—(Duplicate of jar No. 7.) One plant was dead and the other was almost dead. A large number of *Fusarium moniliforme* spores was found on the stem and throughout the jar. The plants in both jars had good root growths.

Jars No. 9 and 10, controls.—The plants in both jars were healthy and apparently doing well. (The plants in one of the jars grew until May 2, when it was removed to make room for another jar; the plants in the other jar were in a healthy condition until June 8, at which time the work was discontinued.)

In the second series of experiments eight jars were used. Two were inoculated with *Diplodia zeae*, two with *Fusarium moniliforme*, two with *Gibberella saubinetii*, and two were held as controls. This series was planted March 28 and the first notes were taken on April 11. At that time one plant was dead in each of the *G. saubinetii* jars and one was dead in one of the *F. moniliforme* jars. The plants in all other jars were growing well and showed no noticeable difference from the control. On April 25 the jars were again examined. The controls were growing splendidly. The *F. moniliforme* jars showed much improvement and no further effects of the inoculation were visible. The *D. zeae* jars showed the effects of the inoculation. The plants were a little smaller than the controls and had begun to wilt. (See Pl. 2, A, B, and C.)

The third and last series of inoculations was begun on May 5. There were twelve plantings in this series. Two jars were inoculated with *Diplodia zeae*; two with *Fusarium moniliforme*; two with *Gibberella saubinetii*; two with the so-called *Cephalosporium sacchari* from the United States (the fungus found by Manns and Adams (10) so plentifully in seed corn and tentatively referred by them to *C. sacchari*); and two with *Cephalosporium sacchari* Butler from India. The cultures of *C. sacchari* used in the two last-mentioned jars were isolated by Dr. F. J. F. Shaw, Pusa, India, and forwarded to the senior author by Dr. E. J. Butler of the Imperial Bureau of Mycology, Kew Gardens, England. This culture when examined was found to be a *Fusarium*. Doctor Butler forwarded a second culture from Doctor Shaw, which when examined was also found to be a *Fusarium*.

Two of the plantings were used as controls. (See Pl. 2, D, E, F, and Pl. 3, C, D, taken May 16; Pl. 3, A, B, and Pl. 4, A, B, D, taken May 30.)

The results obtained from this work would indicate that certain of these fungi have a more pronounced pathological effect on the young plant than do others; especially when infection comes by way of the soil rather than through the seed. *Fusarium moniliforme* produced but little effect on the growth of the plants; even after the third week very little difference could be noticed between the inoculations and the controls (Pl. 2, A, D, and Pl. 3, A).

*Gibberella saubinetii* produced a distinct pathological effect on the seedling plants. It is quite evident from this study that this fungus must be one of the active factors in producing a poor stand (Pl. 2, B, E, and Pl. 3, B). Those plants which survived the seedling stage recovered somewhat and continued their growth, although considerably stunted. In this series the principal effect of *Diplodia zeae* was apparently a retarding of the seedlings in the early stages of growth. The inoculated plants when not killed were much smaller than the controls (Pl. 2, C, F, and Pl. 4, A).

The organism referred to as *Cephalosporium sacchari* by Manns and Adams (10) gave no indication whatever of injuring the plants (Pl. 3, C, and Pl. 4, B). In all of the inoculations with this organism the plants were equal in growth and appearance to the controls.

The culture which Doctor Butler forwarded from Doctor Shaw of India as *Cephalosporium sacchari* Butler, was somewhat active as a corn parasite in the inoculations; it behaved somewhat like *Gibberella saubinetii* in destroying seedlings and retarding growth (Pl. 3, D, and Pl. 4, C).

#### SUMMARY

(1) Cultures of the fungous flora of soil from a field showing much rootrot failed to give any of the so-called rootrot organisms. Laboratory studies on this particular soil gave better response with corn than soil from the adjacent field which in 1921 showed no rootrot. These studies indicate that corn rootrot on the field which gave poor results in 1921 was induced by factors other than the so-called rootrot fungi. Probably a combination of poor drainage and lack of fertility were the chief factors.

(2) In these investigations it has been possible by careful selection and surface disinfection to obtain seed corn sufficiently free internally from disease for experimental work.

(3) *Gibberella saubinetii* proved to be the most active seedling parasite of corn and may be an important factor in reducing stands.

(4) In these investigations *Fusarium moniliforme* appeared less active as a seedling parasite than *Gibberella saubinetii*.

(5) *Diplodia zeae* is quite active in retarding the growth of the young plants. Inoculated plants were much inferior to the controls.

(6) The organism so abundant in corn and referred to as *Cephalosporium sacchari* Butler by Manns and Adams (10) showed no pathogenicity whatever in this study.

(7) The organism sent by Doctor Shaw from India as *Cephalosporium sacchari* Butler, which in the writers' hands proved to be a *Fusarium*, was somewhat active as a seedling parasite of corn, when used in infection work.

(8) Corn plants, provided they are properly cared for, may be grown almost to maturity in one-quart Mason jars. They will not reach the size of plants in the field, but will remain vigorous and green (Pl. 4, D).



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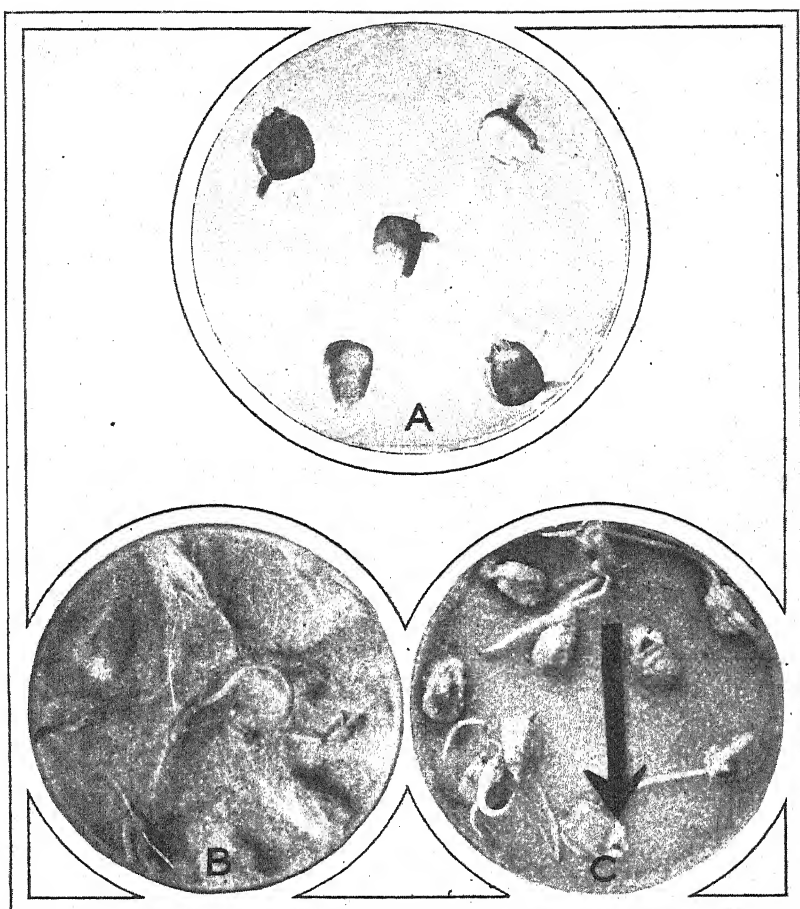


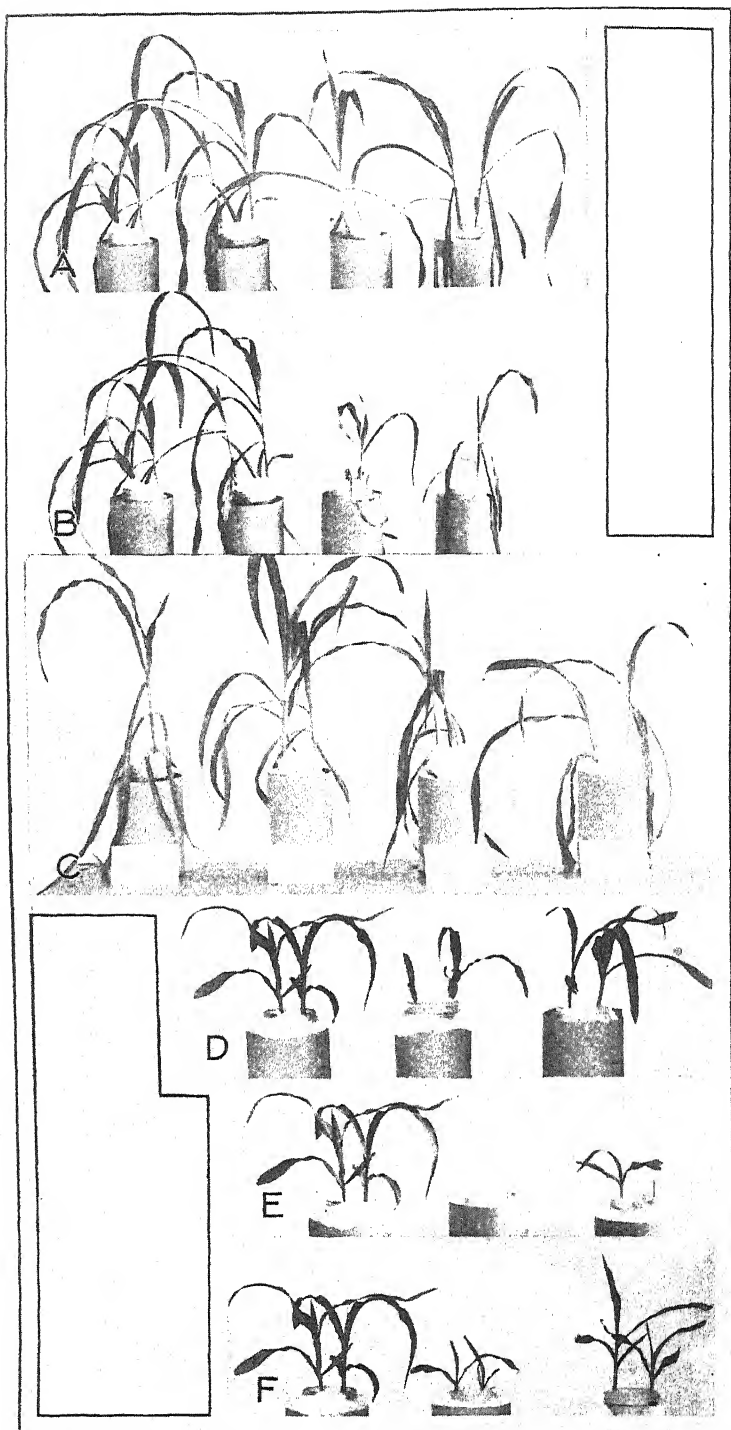


PLATE I

A.—Disease-free seed corn germinated on nutrient glucose agar in Petri dishes. The kernels were surface-sterilized with alcoholic bichlorid of mercury for one minute. (See p. 959.)

B and C.—Internally infected seed corn germinated on nutrient glucose agar in a Petri dish. The kernels were surface-sterilized with alcoholic bichlorid of mercury one minute. (See p. 959.)





## PLATE 2

A.—Four jars in the second series. Corn plants 23 days old. The two jars on the right were inoculated with *Fusarium moniliforme*, the two on the left were controls. There was very little difference in favor of the controls.

B.—Four jars in the second series. Corn plants 23 days old. The two jars on the right were inoculated with *Gibberella saubinetii*, the two on the left were controls. Some strains of *G. saubinetii* appear to be very active factors in destroying seedlings and inhibiting growth.

C.—Four jars in the second series. Corn plants 28 days old. The two jars on the right were inoculated with *Diplodia zeae*, the two on the left were controls. *D. zeae* is apparently an active factor in destroying seedlings and in inhibiting their growth.

D.—Three jars in the third series. Corn plants 11 days old. The two jars on the right were inoculated with *Fusarium moniliforme*, the one on the left was a control. The results indicate *F. moniliforme* to be somewhat active.

E.—Three jars in the third series. Corn plants 11 days old. The two jars on the right were inoculated with *Gibberella saubinetii*, the one on the left was a control.

F.—Three jars in the third series. Corn plants 11 days old. The two jars on the right were inoculated with *Diplodia zeae*, the one on the left was a control. In this series the seedlings were not killed, but the growth was greatly inhibited.

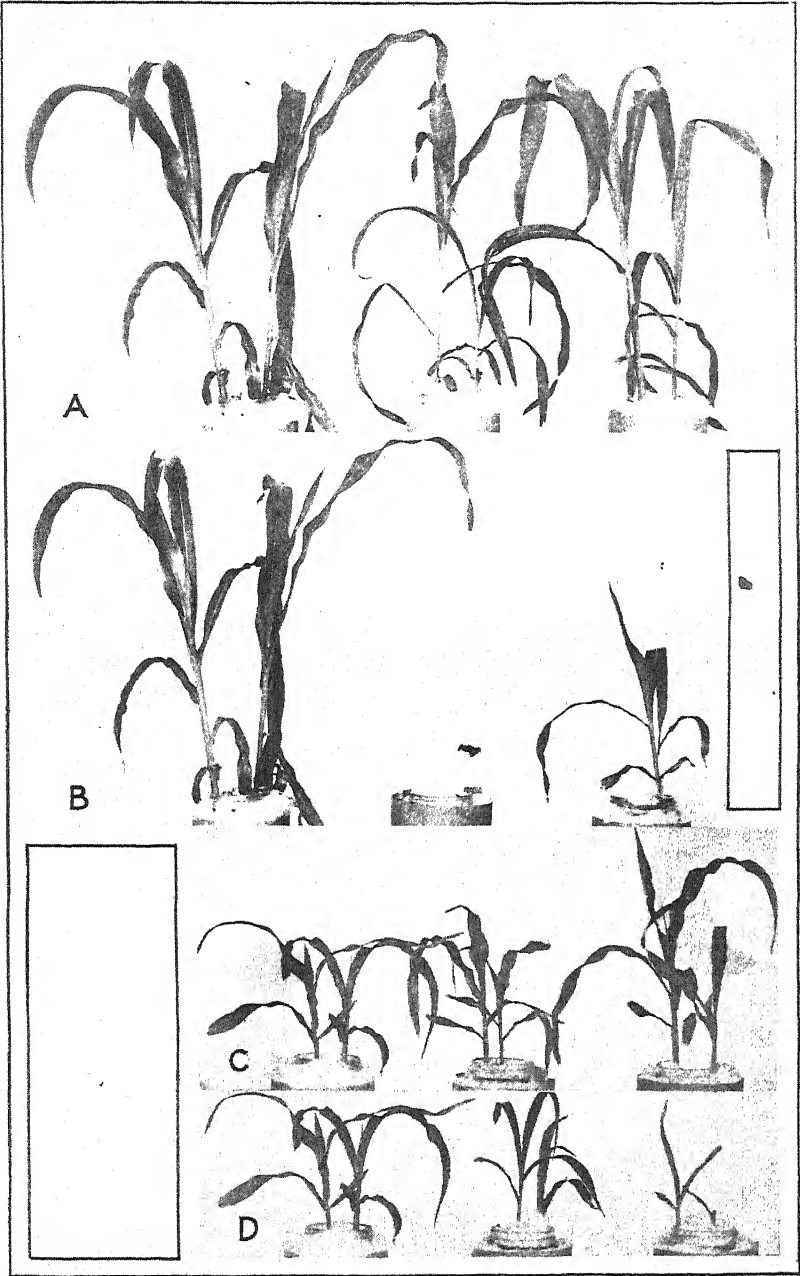
### PLATE 3

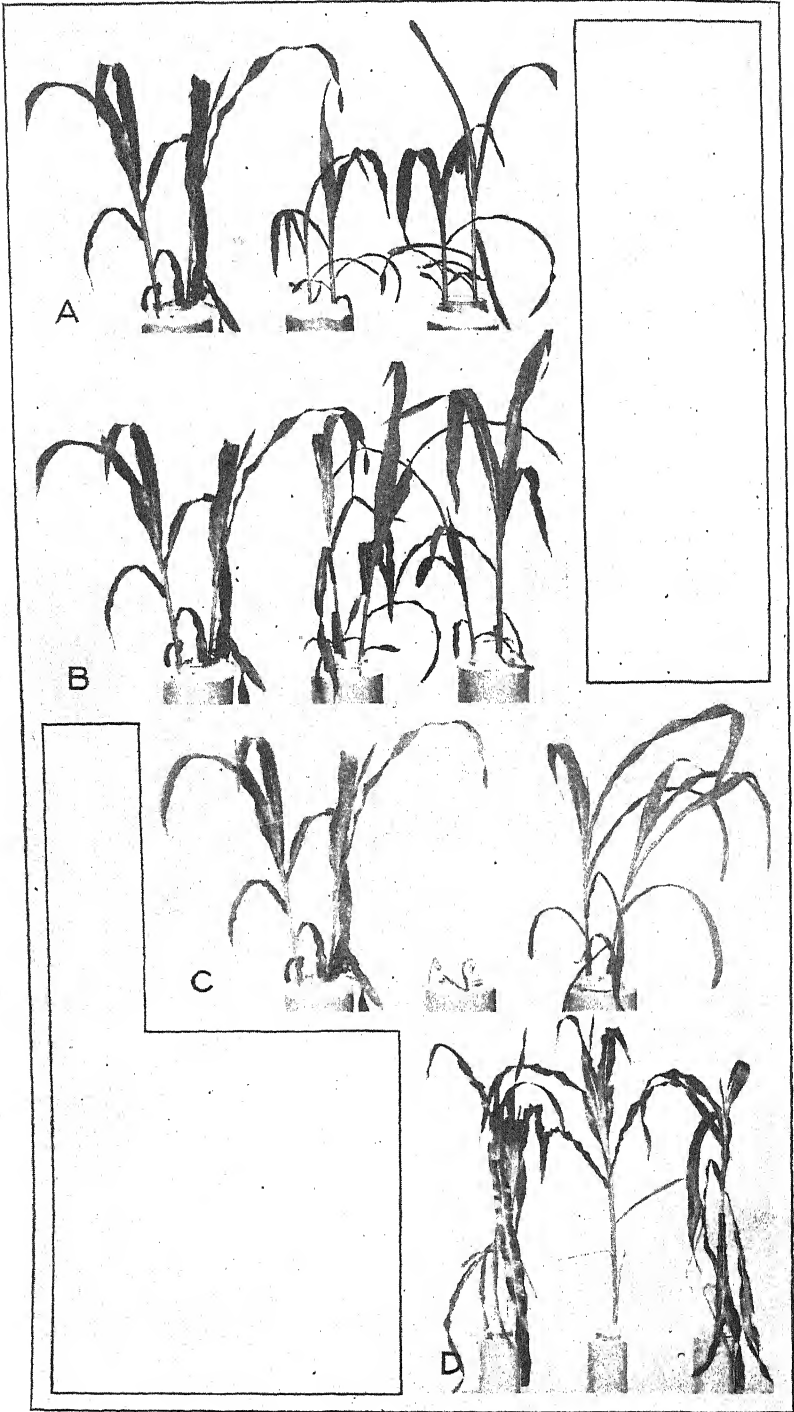
A.—The same jars as those illustrated in Plate 2, D. The corn plants were 27 days old. The growth of the plants appeared to be inhibited to a slight extent by *Fusarium moniliforme*.

B.—The same jars as those illustrated in Plate 2, E. The corn plants were 27 days old. Only one plant was alive in the jars inoculated with *Gibberella saubinetii* and it made very slow growth. *G. saubinetii* appeared to be the most active of the several pathogenes with which experiments were made.

C.—Three jars in the third series. Corn plants 11 days old. The two jars on the right were inoculated with the fungus so prevalent in seed corn in the United States and which was tentatively referred to the species *Cephalosporium sacchari* Butler by Manns and Adams; the one on the left was a control. *C. sacchari* in these experiments apparently had no effect on the seedling plants, since no difference is noticeable between those inoculated and the control.

D.—Three jars in the third series. Corn plants 11 days old. The two jars on the right were inoculated with *Cephalosporium sacchari* Butler, the organism forwarded by Dr. Butler from Dr. Shaw, Pusa, India, to the senior author; the one on the left was a control. This organism, which proved to be a *Fusarium* in the hands of the writers, apparently has an inhibiting effect on the growth of corn seedlings and may destroy them.







#### PLATE 4

A.—The same jars as those illustrated in Plate 2, F. The corn plants were 27 days old. *Diplodia zeae* appeared to cause a marked inhibition of growth.

B.—The same jars as those illustrated in Plate 3, C. The corn plants were 27 days old. The fungus referred by Manns and Adams to the species *Cephalosporium sacchari* Butler has caused no noticeable ill effects except in one plant which appeared to be somewhat weakened in growth.

C.—The same jars as those illustrated in Plate 3, D, showing the work of *Cephalosporium sacchari* Butler, cultures of the fungus direct from India. The corn plants were 27 days old. The plants in one jar have died. The plants in the inoculated jar at the right have almost recovered from the setback received in the seedling stage.

D.—This photograph was taken when the corn plants were about 62 days old. They were growing well and had a good color. This shows that with proper handling corn plants may be grown to a considerable size in jars as small as a Mason quart jar. The plants on the right were inoculated with the fungus referred to tentatively as *Cephalosporium sacchari*. The plants in the jar at the left were controls and received no inoculation.

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